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Studies on growth and reproduction in the rat: I, The value of different cod liver oils for reproduction; II, The value of certain individual foods as sources of vitamins B and G for growth, reproduction and lactation

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STUDIES ON GROWTH AND REPRODUCTION IN THE RAT

(1) The Value of Different Cod Liver Oils for Reproduction

(2) The Value of Certain Individual Foods as Sources of Vitamins B and G for Growth, Reproduction and Lactation

By

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INTRODUCTION

All organisms require food. Food has been defined as "that which is eaten or drunk or absorbed for nourishment". Physiological and Nutritional Chemistry deals with the chemical composition of foods, their special significance and transformations in the organism and with the chemical composition, colloidal nature and properties of the organism itself. The nutritional phase of this subject deals with the food requirements for normal growth, reproduction, lactation and the general welfare of the organism.

The selection of food first depended upon chance and appetite. Those foods readily available, which tasted best, were the foods consumed by the animal. As civilization progressed and governments became concerned with the health of their armies and navies, these governments launched certain planned investigations of epidemics in their military organizations. These investigations indicated that the proper selection of food was necessary for the maintenance of health. The economic importance of any improvement in the health of domestic animals stimulated some study of the food needs of such animals. These studies again pointed to the importance of a satisfactory diet to maintain health and vigor. Subsequent work showed that foods contained proteins, fats, carbohydrates, minerals and water and that all these were required by the animal. The obvious necessity for knowledge concerning the value of different foods in respect to these constituents stimulated the next development in nutrition. Investigators in Germany and in the United States analyzed an enormous number of different foods, compiling tables showing their content of fat, carbohydrate, protein, minerals and water.

The next phase of nutritional investigation involved the biological method of testing foods. Different foods showing identical chemical composition failed to prove equally valuable when incorporated into the diet of the animal. It became apparent that these foods differed in some manner which chemical analysis failed to show. At first these studies were conducted using farm animals as subjects for experiment. This proved entirely too expensive because purified diets were necessary and the large animals employed consumed vast quantities of food. This problem was solved with the discovery that rats showed practically the same reaction to deficient diets that larger animals showed. Rats would consume almost any foods offered to them. The life span of the rat was short and permitted rapid determination of the effects of different diets. It may well be said that real progress in nutrition followed the discovery that the rat could be used as an experimental animal.

Successful growth on a given diet with failure to grow on another diet which was apparently identical chemically, caused various investigators to postulate the existence of accessary food factors which were necessary for satisfactory growth. Several of these factors have been discovered and are called vitamins. Similar experiments have shown the necessity of certain minerals in the diet and have modified our conception as to the necessity of proteins, carbohydrates and fats. Certain recent conceptions regarding the accepted dietary requirements are summarized in the following paragraphs.

Vitamin A. Vitamin A is necessary for normal growth and reproduction. Deficiency of this factor in the diet results in keratinization of the epithelial tissues. A secondary infection often develops in the eye. This disease of the eye is called Xerophthalmia. Prolonged deficiency of vitamin A causes failure of ovulation in the female and testicular degeneration in the male. This male sterility is temporary while male sterility due to a lack of vitamin E is permanent. Recent work indicates that nerve degeneration is characteristic of vitamin A deficiency.

Certain animals are able to convert carotene into Vitamin A. The suggestion has been made that the vitamin is an alcoholic derivative of carotene with about one half the molecular weight of the carotene molecule. Highly active concentrates of the vitamin have been obtained in at least three different laboratories, using different sources as starting materials. These preparations are so similar that we may assume they contain the vitamin in a fairly high state of purity.

Vitamin B. Early investigators assigned the name vitamin B to what is now known as the B complex. What was formerly considered the vitamin has now been shown to contain two factors. Suggestions in the current literature indicate that these may each contain two factors themselves. This would subdivide the original B complex into four factors. The two factors admittedly present in the B complex are vitamin B (B_1) and vitamin G (B_2). American workers use the terms B and G while the English workers use the terms B_1 and B_2. The heat labile, appetite stimulating and antineuritic factor is called vitamin B (B_1). The heat stable, growth promoting and pellagra preventative factor is called G (B_2).

Recent work has shown that vitamin G (B_2) probably consists of two factors. One factor is a growth promoting flavin while the second factor protects against pellagra. Other workers have suggested that the B complex contains an additional factor called B_4. This factor is prepared from concentrates of vitamin B_1 and is absent from alkali-autoclaved marmite. It is heat labile in alkaline solution. The physiological effect of B_4 is similar to that of B_1 and many workers say these two factors are identical. The designation B_3 has been applied to various factors reported present in the B complex. Conclusive evidence of their existence is lacking.

Deficiency of vitamin B (B_1) results in a disease called beri beri in man and polyneuritis in animals. These diseases are characterized by nerve degeneration, failure of reproduction and lactation, loss of appetite and loss of tonicity of the digestive tract. Deficiency of vitamin G (B_2) results in failure of growth and the development of skin lesions. This disease is called pellagra.

Vitamin C. In the absence of this vitamin scurvy develops in man, monkey and the guinea pig. Rats, birds and dogs either do not need the vitamin or are able to synthesize it. This vitamin has been synthesized in various laboratories and is obtainable from pharmaceutical houses. Recent work indicates that vitamin C is essential for proper bone development and the maintenance of the teeth.

Vitamin D. This vitamin is essential for the proper development of bones and teeth. In its absence a correct ratio of calcium to phosphorus must be maintained to prevent the disease called rickets. The mechanism by which the vitamin enables the body to correctly utilize the calcium and phosphorus, even when their relative amounts in the diet are not optimum, is not yet clear. The vitamin is produced in animals by exposure to ultraviolet light. The irradiation of ergosterol produces vitamin D. The active material can be separated from this irradiated ergosterol and crystallized to form a more economical source of the vitamin than cod liver oil. Recent work has shown that cholesterol can be treated with sulfuric acid in the presence of a dehydrating agent to produce a substance which possesses a higher curative action upon rickets in chickens than an equal amount of irradiated ergosterol.

Vitamin E. This factor is called the anti-sterility vitamin. In its absence the female resorbs the foeti before the young come to term and the male becomes permanently sterile due to degeneration of the germinal epithelium. Deficiency of this vitamin in the diet of the lactating mother results in the development of a peculiar type of paralysis in the suckling young. This paralysis is especially noticeable in the posterior extremities and develops during the third or fourth week of lactation.

Vitamin F. Recent work suggests that normal reproductive function requires certain unsaturated fatty acids in addition to vitamin E. Fat free diets do not permit reproduction and if saturated fats are incorporated into such diets they still fail to permit reproduction. When certain unsaturated acids are added to these diets, the animals are able to reproduce but lactation is still below normal.

Vitamin G. This vitamin was discussed under the head of vitamin B.

Minerals. The mineral requirements of the animal have not been completely established. The literature is contradictory or inconclusive in many instances. General agreement exists at present that certain elements are essential in the diet. These elements are hydrogen, potassium, sodium, magnesium, calcium, copper, iron, carbon, nitrogen, oxygen, sulfur, phosphorus, chlorine and iodine. Further investigation will undoubtedly show that other elements are essential. The extreme difficulty of preparing diets free from the element to be tested and the difficulty of obtaining the required element in the pure state both contribute to the slowness of progress in this field of investigation.

Fats. Fats provide an excellent source of energy for the animal. During normal metabolism the glycerol of the fat molecule may be converted into glucose to augment the supply of carbohydrate. It is still an open question as to whether the fatty acids are ever converted into carbohydrate. Some investigators maintain that fat is not an indispensable component of the diet. Recent work indicates that certain unsaturated fatty acids are essential and must be included in the diet.

Carbohydrates. Carbohydrates provide another source of energy for the body. It is true that the glycerol portion of the fat molecule may be converted into glucose, but the absolute exclusion of carbohydrates from the diet results in the improper metabolism of fats with an attendant ketosis. This has been attributed to the flooding of the metabolic mill with fat to a point where the organism is unable to handle this source of energy.

Proteins. Proteins constitute the most expensive portion of the diet of the animal. For this reason proteins must be selected with care because different proteins are often very different in their dietary value. Higher animals are unable to synthesize certain essential amino acids which are present in the protein molecule and proteins should be selected on the basis of their content of such amino acids. Proteins can be converted into carbohydrates or fats but economic considerations preclude their use for such purposes.

REVIEW OF LITERATURE

The literature concerning nutrition is entirely too voluminous for a complete review in this paper. No effort has been made to present an exhaustive review of the literature concerned with reproduction and lactation. Only the literature which has a direct bearing on the study reported in this paper will be mentioned. Certain books may prove helpful in obtaining a general view of the subject. The reader is referred to "The Newer Knowledge of Nutrition", by E. V. McCollum and Nina Simmonds (34), "The Vitamins", by H. C. Sherman and S. L. Smith (63) and "The Vitamins", a symposium, reprinted by the American Medical Association for Mead Johnson and company of Evansville, Indiana, and distributed by this company (41).

Satisfactory reproduction in animals is of immense practical and economic importance. Previous to 1922 many workers had been unable to obtain satisfactory reproduction on diets of purified foods. Evans and co-workers (1927)(15) had observed sterility due to the failure of the normal development of the foetus in the female and of the seminiferous epithelial cells of the male rat when maintained on a diet of the following composition: casein 18%, corn starch 54%, lard 15%, butter fat 9%, salts (McCollum) 4% and Brewer's yeast (Harris) 0.4 - 0.6 grams per rat per day. Closer examination of the female revealed that the oestrus cycle was normal, conception and implantation proceeded as usual but that on the sixteenth day of gestation the foeti died and were resorbed. The sterility in the female was temporary and could be overcome by feeding vitamin E. Sterility in the male was permanent and proceeded in four stages. First, there is a normal amount of sperm but loss of fertilizing power. Second, there is a complete loss of sperm. Third, there is loss of the power to form the vaginal plug. Fourth, there is complete loss of all sex interest.

Further investigation by these workers proved that increasing the amount or quality of protein, carbohydrate or of vitamins A, B, C or D did not bring better results. Feeding lettuce, wheat, wheat germ, meat or oats corrected the difficulty and permitted normal reproduction. They designated the unknown constituent factor X. Sure (1924)(68, 69) using milk protein diets corroborated the existence of the vitamin and Mattill and Stone (1923)(40) accepted the lack of vitamin E as an explanation of their failure to obtain satisfactory reproduction on milk diets. Sure (1924)(69) proposed the name vitamin E and contributed much valuable information (70, 71, 72, 73, 75) concerning its properties and distribution. Other workers accepted vitamin E as the name of the new factor. Hogan and Harshaw (1924)(24) reported that they had obtained fourth generation rats without any known source of vitamin E in the diet unless butter fat contained the vitamin. These investigators later (1926)(25) admitted the existence of the vitamin and verified Evans' results on the characteristics of male and female sterility in its absence. Kennedy (1926)(30) found that vitamin E was essential for normal reproduction.

Nelson and co-workers (1923)(50) reported rearing third generation young on diets in which vitamins B and G were supplied by 5% of yeast. This diet contained no known source of vitamin E and although the rats in the second and third generation did not grow normally the trouble was not in reproduction. Anderegg (1924)(3) reported normal growth and reproduction with diets containing whole milk as the only source of protein and vitamins. His problem was one of lactation and he considered it unnecessary to assume a reproductive vitamin. Nelson and co-workers (1925)(51) obtained better reproduction and lactation on diets containing cane molasses as the sole source of vitamin B than they had previously obtained with diets in which yeast supplied the vitamin. The molasses diet contained no known source of vitamin E.

Anderegg and Nelson (1925)(4) repeated and verified the work of Mattill and Stone with diets including whole milk powder and lard. These diets resulted in sterility but removal of the lard gave normal reproduction. Anderegg and Nelson (1925)(4) next supplemented skim milk with 5% of butter fat or 5% of cod liver oil to make it comparable to whole milk. Sterility resulted on these diets but when they were supplemented with 8% of extracted wheat embryo or 5% of air dried yeast the diets permitted reproduction for one generation only.

Anderegg and Nelson (1926)(5) noticed that when they incorporated cod liver oil into the skim milk diet in kilogram lots that a very pungent and penetrating odor resulted which was suggestive of acrolein. This decomposition did not occur if wheat embryo oil was also added. They further noted that the decomposition was prevented by the addition of water or alcohol. When 10% of water was added and the diet allowed to stand for several months the absence of the odor indicated that no decomposition had taken place. Fourth generation animals were reared on skim milk diets if 5% of water was added and the cod liver oil was fed daily. The authors did not deny the existence of a vitamin for reproduction but their data did not prove the necessity for such a vitamin. Nelson and co-workers (1926)(53) found that rats would tolerate a high percentage of butter fat but would not reproduce on diets containing a high percentage of lard. They noted that 12% of yeast gave better results than 5% of yeast and concluded that the limiting factor for reproduction and lactation was vitamin B. Mattill and co-workers (1924, 1926)(38, 39) repeated the experiments of Nelson and Anderegg using 50% of whole milk powder with no additional fat. They verified the results of these investigators and further showed that when 15% of fat was added to such a diet the animals were sterile. They suggested that high fat diets might increase the need for vitamin E and made the further suggestion that butter fat contained vitamin E.

Evans and co-workers (15) reported sterility in animals fed diets containing 22% of lard and 2% of cod liver oil. They stated that cod liver oil was free of vitamin E. In their work they had used oils purchased from Mead Johnson and company, and from the wholesale druggists, Coffin and Redington, and Langley and Michaels, both of San Francisco. More recently they had used an oil purchased from E. L. Patch and company which contained no vitamin E. Sure (1927)(76) used cod liver oil purchased from the Sea Pure Products company, of Portland, Maine. He reported that cod liver oil contained no vitamin E. Nelson and co-workers (1927, 1928, 1930)(52, 54, 82) had used Squibbs cod liver oil and had been unable to show that it was free of vitamin E. These workers showed that the oil decomposed when mixed with the diet and allowed to stand for long periods and that it decomposed in the presence of the high percentage of lard used in the diets employed by Evans. These workers further found that Squibbs cod liver oil was superior to butter fat as a source of the fat soluble vitamins. They suggested that the difficulty experienced by other investigators was due to the decomposition of the oil as described above.

During this period many investigators were attempting to determine the existence or the non-existence of vitamin E in different foods. Sure (1927)(77) reported that 10% of butter fat gave continuous fertility but did not approximate the potency of wheat oil in vitamin E. Simmonds, Becker and McCollum (1928)(64) reported that vitamin E was present in the cod liver oil and the lard they had used. They used cod liver oil obtained from Mead Johnson and company and found that diets containing

22% of lard and 2%, 3% or 4% of cod liver oil gave better fertility than diets containing 22% of lard and either 5% or 10% of butter fat. Wilkinson (1930)(85) investigated the effect of lard on various diets. He used a diet containing casein 18%, extracted wheat embryo 10%, salts (185) 3.7%, Squibbs cod liver oil 5% and enough dextrin to make up 100%. He showed that reproduction was normal if the cod liver oil was mixed into the diet daily. He now modified the diet replacing 15% of the dextrin with 15% of lard. If he mixed the cod liver oil into this new diet in kilogram lots the animals did not reproduce. He modified his technique and mixed the cod liver oil into the diet forty eight hours before use. In this experiment three females produced five litters and two of the females died at parturition. The mortality of the young was 100%. The next modification of technique involved the mixing of the cod liver oil into the diet daily. In this experiment four females each produced four litters but lactation was poor. A further modification of the diet involved the replacement of the lard with lard which had been autoclaved for one hour at fifteen pounds pressure. The cod liver oil was mixed into this diet in kilogram lots., On this diet three females each had two litters, one female had one litter and another female had three litters. Two females died in parturition after having two litters. The mortality of the young was 67%. Evans (1932)(14) reported that lard contained a certain substance, or substances, which inactivated the vitamin E in wheat germ.

Recent work by Mattill may serve to reconcile the divergent views concerning the presence or absence of vitamin E in different foods. Mattill investigated the suggestion of Greenbank and Holm (1924)(20) that water vapor retarded the auto-oxidation of butter fat. He found (1927)(37) that cod liver oil was more readily auto-oxidized than butter fat. He believed that butter fat contained vitamin E which would permit normal reproduction if properly protected. He found that oxidative changes which accompany rancidity in unsaturated animal fats tended to destroy vitamins A and E. This oxidative action was delayed by the presence of hydroxyl groups and wheat oil contained more of these groups than lard, cod liver oil or butter. This might explain the protection of the vitamin in wheat oil. Evans (1932)(14) reported a private communication from Mattill which stated that Squibbs cod liver oil is the least prone to oxidative changes. Cummings and Mattill (1930)(8) found that cod liver oil was most susceptable to oxidation, while lard was second and butter was third. A combination of mixed fats would influence the auto-oxidizability of the mixture. Lard and cod liver oil would thus be a combination very susceptable to destruction of vitamin E by oxidation. They further stated that our knowledge of the sources of vitamin E was relative and not absolute and the efficiency of a given source depended on the auto-oxidizable materials and anti-oxidants associated with it. Olcott and Mattill (1931)(55) obtained a crystalline anti-oxidant from lettuce. They considered that this was proof that the vitamin and anti-oxidants were different substances.

Evans and co-workers (15) reported that vitamin E was characterized by other functions than its effect on the reproductive system. Evans and Burr (1928)(17) reported that vitamin E was secreted by the mammary gland. If the mother did not obtain sufficient vitamin E during the lactation period the young developed paralysis of the musculature of the body. The paralysis appeared in the posterior extremities between the eighteenth and twenty fifth day of life. About 35% of such young died and about 48% of the young which survived carried some muscular paralysis throughout life. This disorder was prevented by feeding larger amounts of vitamin E to the mother. Evans (1928)(13) reported that later growth phases were impaired if the rats were maintained on diets free from vitamin E. Sure (1926)(74) reported that the vitamin E complex contained two factors. One factor promoted lactation while the other had anti-sterility properties. The lactation factor was thermostable and the anti-sterility factor was thermo-labile. Both were present in wheat germ oil. Sure (1926)(72) reported that wheat oil, cotton seed oil, corn oil and palm oil contained vitamin E and also permitted normal lactation. Peach kernel, soy beans, peanut oil and oil of the olive contained vitamin E but did not permit normal lactation. Tso (1927)(83) reported that a lactation factor might be present in a vitamin E rich food. The diet used consisted of casein, corn starch, yeast, salts, 5% of butter fat and 1% of lard. He stated that reproduction occurred on this diet but that the mortality was high. The addition of fresh lettuce resulted in successful lactation. Moore (1926)(44) found that Squibbs cod liver oil was superior to butter fat in promoting either reproduction or lactation. Diets containing 16% of extracted wheat embryo plus 5% of butter fat gave 100% mortality while the same diet with Squibbs cod liver oil replacing butter fat permitted the rearing of all the young. Morelle (1931)(45) confirmed the observation of Evans concerning paralysis of the nursing young due to a deficiency of vitamin E in the diet of the mother. He reported that wheat germ oil was a successful prophylactic even when the treatment was not started until five days after the birth of the litter. Feaster (1934)(18) found a higher mortality and a lower weaning weight on diets containing 30% wheat and 5% of butter fat than he found on diets containing 30% of wheat and 4% of butter fat plus 1% of Squibbs cod liver oil.

All comparative studies using the rat as the experimental animal must either assume that these animals show the same behavior at different seasons of the year or a suitable correction must be made or a suitable control provided. The literature concerning the normal behavior of the rat in different laboratories is not entirely concordant. Donaldson (10) stated that under normal conditions rats breed in all seasons of the year but breed most readily in the spring. She stated that constancy of moderate temperature was usually favorable to continuous reproduction. King and Stotsenburg (1915)(32) reported that the rat showed no restricted breeding season but that under similar environmental conditions other than temperature more litters were produced in the spring and autumn. These investigators observed a seasonal variation in sex ratio but no seasonal variation in litter size. Hogan and Harshaw (1924)(24) reported that fewer litters were born in their colony during the winter and that a proportionately smaller number was reared. The number reared varied between 13% and 91%. They made no mention of controlled conditions. Hanson and Sholes (1924)(23) reported that they maintained uniform laboratory conditions. The temperature rarely reached the extremes of fifteen and twenty seven degrees centigrade and the laboratory was well ventilated. There was no seasonal statistical variation in birth weight, litter size or sex ratio. In the spring the birth weight was actually highest and the sex ratio was actually the lowest while the sex ratio was actually the highest in the winter. These variations did not require a correction factor on experimental data.

Hanson and Heys (1924)(22) reported seasonal variation in the growth of females under uniform laboratory conditions. Females born in the fall months showed the greatest mean body weight at all ages while those born in the summer showed the lowest mean body weight. King (1924)(31) reported a seasonal variation in litter production of the less domesticated Norway strain of rats. More litters were born during spring and summer than during autumn and winter. The fewest litters were born during the autumn months. She found that the physical condition and age of the female were the most important factors for litter production. Strain became a factor when it involved degree of domesticity. Smith and Bing (1928)(67) working in Mendel's laboratory at Yale, reported that rats born in winter grew at a slightly slower rate than the colony curves but were well within the limits of the standard deviation of the colony. These investigators stated that temperature, humidity, light and sanitation were of the utmost importance. These factors were carefully controlled in their laboratory. Orent and McCollum (1931)(56) stated that five experimental rats on a manganese free diet died during the summer of 1930. They attributed the deaths to the heat because thirty one of the stock rats died during one hot night of that summer. Bills and co-workers (1931)(6) reported no seasonal variation in fertility. Bills' laboratory was very carefully controlled. He used rats of the inbred Wisconsin variety as used by McCollum and Steenbock. He kept the rats in metal cages and bedded them in sterile shavings. The temperature was held between twenty and twenty five degrees centigrade throughout the year. The breeders were selected by weight from young weaned each week so that the age average of the parents was constant.

The literature concerning the vitamin B complex and the subsequent recognition of the non-identity of the various factors present in this complex has been reviewed in many papers. Four of these papers are mentioned because they are rather significant or complete. Mitchell (1919)(42) reviewed the early phases of this work. Lewis (1933)(33) and Simonnet (1933)(65, 66) presented adequate reviews of subsequent work.

Various investigators have suggested that satisfactory lactation required increased amounts of the vitamin B complex. Adair (1925)(1) presented a review of the literature concerning humans and laboratory animals and concluded that the vitamin content of the milk depended upon the vitamins ingested. Sure (1924)(70) suggested that the requirements for the vitamin B complex in lactation were much greater than for growth. Sure (1928)(78, 79) made a quantitative study of the requirements for the vitamin B complex in lactation and concluded that three times the amount of yeast necessary for growth was required by the lactating mothers. He stated that the lactating mother was about 40% efficient. By feeding a portion of the yeast directly to the young he could rear them with a smaller yeast supplement. Nelson and co-workers (1926)(53) used yeast as the sole source of the vitamin B complex and reported that three to five times as much yeast was required for lactation as for growth.

Later investigations concerned the two known factors of the vitamin B complex. Sure and Walker (1931)(81) studied the lactation requirements for the vitamin B of the B complex. They restricted the food intake and kept everything constant except the vitamin B. Rearing of young was unsuccessful with small amounts of vitamin B but was successful with larger amounts of the vitamin. They concluded that vitamin B was essential for lactation. Sure (1928)(80) reported that the vitamin G requirement was three times greater for lactation than for growth and that the vitamin B requirement was also greater for lactation than for growth. Hussemann and Hetler (1931)(29) reported that lactation increased the requirements for both vitamins B and G but they considered vitamin G the more important. Moore and co-workers (1932)(43) studied first and second litters of mothers raised on diets containing both vitamins B and G. They concluded that another factor was required for successful lactation. They suggested that this was probably Reader's (58, 59) vitamin B_4. Feaster (1934)(18) reported that normal lactation required six times the amount of both vitamins B and G required for normal growth.

No specific factor has been accepted as essential for lactation. It was pointed out above that considerable amounts of vitamins B and G were required and that contamination of the preparations of vitamin B with Reader's vitamin B_4 may account for its significance in lactation. Nakahara and co-workers (1934)(49) claimed to have found such a factor in beef liver. They called it factor L. This factor was present in the non-adsorbate fraction of fresh beef liver which had been treated with acidified alcohol and white acid earth. The basal diet of Nakahara (1933)(47) consisted of polished rice 75 grams, fish protein 10 grams, butter 5 grams, salts (185) 5 grams and dried yeast 5 grams. On this diet growth was normal but mortality was practically 100%. Nakahara and Inukai (1934)(48) reported that the addition of three grams of their liver extract which was equivalent to one hundred grams of fresh liver would permit normal lactation if this amount was added to each one hundred grams of the basal diet.

Comparatively few investigators have reported on the vitamin B and G content of the specific foods studied by the author. Still less work has been done showing the connection between the vitamin B and G content of these foods and their relation to lactation. Guest, Nelson and co-workers (1926)(21) reported that diets containing 10% or 15% of wheat as the sole source of the vitamin B complex gave normal growth but did not permit the rearing of normal young. Diets containing 35% of wheat permitted reproduction through the fourth generation but did not permit normal lactation. Failure of lactation was shown by the high mortality of the young. Wilkinson (1930)(85) reported that diets containing 30% of wheat as the sole source of vitamins B and G did not permit satisfactory lactation, as shown by 32% mortality after the seventh day. When 25% of liver was added to the diet, the mortality of the young fell to 8%. Sherman and Axtmayer (1927)(61) reported that vitamin G was the limiting factor for growth on diets containing whole wheat. Hunt (1928)(26) reported that 15% of wheat was sufficient for growth while 25% to 35% of wheat was required as the sole source of vitamin B for normal lactation. Aykroyd and Roscoe (1929)(2) studied wheat as a source of vitamin G for growth. They found that 30% of Manitoba wheat was insufficient while 50% of Manitoba wheat gave excellent growth and 50% of English wheat gave normal growth. Taylor (1930) (82) found that wheat did not contain sufficient vitamin G for lactation. Diets containing 73% of wheat showed 57% mortality. This high mortality was reduced by feeding yeast or autoclaved yeast. Feaster (1934)(18) reported a mortality of 55% on diets containing 30% of wheat. The addition of 5% of yeast reduced the mortality to 17% while the addition of 10% of yeast reduced the mortality to 13%. The addition of 10% of autoclaved yeast gave a 17% mortality. He concluded that vitamin G was the limiting factor for lactation on diets containing wheat, since 60% of wheat did not contain enough vitamin G to permit satisfactory lactation.

Osborne and Mendel (1930)(57) fed dried alfalfa as the sole source of vitamins B and G. They fed one half of one gram per rat per day. This amount represented from 5% to 9% of the total diet. Excellent growth resulted and they concluded that alfalfa was an excellent source of the vitamin B complex. Hunt and Krauss (1931)(28) studied the influence of the diet of the cow on the vitamin B and G content of the milk. They found that pasture feed produced a milk of higher vitamin G content than dried feed. The content of vitamin B in the milk was not affected. They suggested that vitamin G was synthesized when the plant was growing vigorously. Douglass (1932)(11) reported that there was a progressive increase in the vitamin B complex from the bud to the one half bloom stage. The first cutting of alfalfa was richest in vitamin B and contained twice as much as the third cutting. Douglass and co-workers (1933)(12) reported that from four tenths to five tenths of a gram of freshly cured alfalfa was required to supply sufficient vitamin B for growth while thirty five hundredths of a gram furnished sufficient vitamin G for growth. This indicated that alfalfa was a better source of vitamin G than of vitamin B. Douglass stated further that such factors as rain, time of harvest, season, curing methods and the age of the plant affected the content of vitamin B. The content of vitamin G was less variable.

Richardson (1916)(60) reported that normal growth and lactation were obtained on diets containing 45% of cotton seed meal supplemented by 17% of whole milk powder. These diets contained 10% of starch and 28% of lard. Hunt (1932)(27) stated that cotton seed meal was a good source of the vitamin B complex for the rat and that 20% was optimum. He found that reproduction was abnormal because gossypol was present. He further stated that 75% of the gossypol was removed in the commercial preparation of the cotton seed meal. Munsell and co-workers (1933)(46) stated that diets containing 30% of cotton seed flour were not toxic to rats when fed for a period of six months. They compared the content of the flour in vitamins B and G to the vitamin content of yeast. Taking the value of yeast to equal one hundred in each of these vitamins they found that the value of cotton seed flour in vitamin B was forty three and in vitamin G was ten. Whitsitt (1933)(84) reported that cotton seed meal was a rich source of vitamin B and a good source of vitamin G. Gallup and co-workers (1934)(19) reported that gossypol could be detoxicated by adding 2% of sodium bi-carbonate plus 2% of calcium carbonate to the diet.

Wilkinson (1930)(85) used 40%, 60% and 73% of powdered butter milk as the sole source of vitamins B and G in synthetic diets. He reported that the diets containing 40% and 60% of the butter milk powder gave normal growth but that all the young died between the fifteenth and eighteenth days. The diet containing 73% of the butter milk powder was poorer for growth and permitted no reproduction.

No work, using rats as experimental animals, was found in the literature concerning the vitamin B and G content of linseed oil meal, fish meal or tankage. Daniel and McCollum (1931)(9) reported that the protein value of fish meal was equal to or superior to the casein control. They also found that tankage was much inferior to any fish meals studied. Braman (1931)(7) reported that 8% of linseed oil meal provided better protein for growth than 8% of cotton seed meal.

EXPERIMENTAL

Statement of the Problem

Different investigators have reported varying results in experiments using cod liver oil as a source of vitamin E. It was the original purpose of the author to investigate different brands of cod liver oil which had been used in these laboratories and determine their relative potency in vitamin E. During the progress of this work it became apparent that differences were so slight in certain cases that other factors became the determinants. The same diet fed at different seasons of the year or fed during the same season in different years would fail to give identical results in studies of reproduction or of lactation. This led to an investigation of the entire stock colony of our laboratory to determine whether seasonal variations were important.

Other investigations in progress in our laboratory indicated that the diet fed to our stock colony might be improved. This diet, hereafter referred to as the growing ration or as diet 47, consists of various natural foods. My problem now expanded to include a study of the potency of certain of these natural foods in vitamins B and G and a further investigation of the value of these foods for lactation.

Sources and Preparation of Materials

Casein.

Casein was used as a source of protein in these feeding experiments. Commercial casein was purchased from the Wilkins-Anderson company of Chicago, Illinois. This casein was purified by washing with distilled water acidified with acetic acid. The washing was done in battery jars and the water was changed daily for three weeks. This washed casein was dried at a temperature of eighty five degrees centigrade.

Dextrin.

Commercial corn starch was obtained from the Penick and Ford company of Cedar Rapids, Iowa. The starch was worked into a stiff paste with thirty seven hundredths per cent citric acid solution. This paste was autoclaved at fifteen pounds pressure for two and one half hours and then dried at eighty five degrees centigrade.

Salt Mixture 185.

McCollum and Simmonds (35) salt mixture 185, fortified with two grams of potassium iodide per three and one half kilograms of salt mixture, was fed at a level of 3.7% throughout these experiments.

Yeast.

Dried yeast, purchased from the Fleishman Yeast company, was employed as a source of water soluble vitamins.

Autoclaved Yeast.

Dried yeast was moistened with enough distilled water to form a thick paste. The paste was placed in glass crystallizing dishes in layers about one inch deep and autoclaved at fifteen pounds pressure for five hours. The product was dried at eighty five degrees centigrade and used to supply vitamin G in certain experiments.

Butter Fat.

College butter was melted in four liter beakers. The water and curd settled to the bottom of the beakers and the fat was filtered through folded filter papers.

Cod Liver Oil.

Samples of various cod liver oils used in this work were sold under the following names: Squibbs, Sea Pure, Des Moines Drug Co., Parke Davis, Fuller-Morrison, Eli-Lilly, Meads, Marden-Wilde. These oils were kept in a cool room in the basement when not in use.

Wheat Germ.

The wheat germ was a very clean product obtained from Washburn and Crosby.

Extracted Wheat Embryo.

Wheat germ was extracted with anhydrous ether in a large continuous extractor for periods ranging between forty eight and sixty hours. The ether siphoned about every hour. The extracted product contained less than 0.3% of ether soluble material.

Alfalfa.

The alfalfa used was a special shipment from the Allied Mills and was obtained from their Omaha plant. The product was described as a finely ground leaf meal of the first and second cutting. It was immature and was artificially dried. The protein content was 19.3%.

Fish Meal.

The product used was obtained from the Atlantic Coast Fisheries and was called Atco fish meal. It was prepared from haddock and contained 65.4% protein.

Tankage.

The tankage was obtained from Swift and Co. and was called Swift's 60% protein tankage.

Wheat, Linseed Oil Meal, Cotton Seed Meal and Butter Milk Powder.

These were commercial products obtained on the local market. The protein content of the linseed oil meal was 36.3%. The cotton seed meal contained 42% protein and the butter milk powder contained 31.8% protein.

Rice Polishings.

Rice polishings were obtained from the Arkansas Rice Growers Corp. Assoc., Stuttgart, Arkansas.

Vitamin B_1 from Rice Polishings.

The extraction of the vitamin was carried out in twelve liter battery jars. Four kilograms of rice polishings were placed in each of four battery jars. Ten liters of 95% alcohol containing 5 ml. of glacial acetic acid were added to the first jar. The mixture was stirred eight or ten times per day and allowed to stand over night. The alcohol from this jar was siphoned into the second jar and enough more alcohol added to make the volume equivalent to that originally present in the first jar. New 95% alcohol was placed in the first jar to make up the volume originally present. The same plan was followed until the alcohol from the first jar had been transferred successively to jars 2, 3 and 4. The alcohol from the last jar was siphoned off and filtered and the residue from the jar was sucked dry on a buchner funnel. This process provided for washing each residue with four portions of alcohol. After the four washings the residue was discarded. The volume of the filtrate from each jar amounted to approximately seven liters.

The filtrate was concentrated to approximately 700 ml. under reduced pressure and at a temperature below fifty degrees centigrade. One liter of distilled water was added and the volume again reduced to 700 ml. A layer of oily material separated at this point and was removed in a separatory funnel. The remaining aqueous layer was treated with a 20% solution of neutral lead acetate to the point of maximum precipitation and allowed to stand for six hours. The mixture was filtered and the residue washed with 100 ml. of water. A 20% solution of sulfuric acid was added to precipitate the lead and the mixture was made faintly acid to congo red with 20% sulfuric acid or 10% sodium hydroxide. Seventy grams of fuller's earth were added to the mixture which was stirred at intervals for three hours. During this time the mixture became more alkaline and acid was added to maintain approximately the same acidity as shown by congo red. The fuller's earth carrying the adsorbed vitamin B was filtered off, air dried, and finally dried in a vacuum desiccator over calcium chloride. This material was used wherever vitamin B is indicated in the experimental work.

Hog Liver.

Hog liver was used for the preparation of the vitamin G concentrate. The liver was obtained on the local market and was worked up as soon as possible after it had arrived from the packers.

Vitamin G from Hog Liver.

One kilogram of finely ground hog liver was stirred into 2000 ml. of boilimg water. The mixture was heated to boiling and the boiling was continued for three minutes. The filtration was accomplished on a Buchner funnel and the residue was washed with 500 ml. of hot water. The filtrate was concentrated to 150 ml. under reduced pressure and 850 ml. of absolute alcohol were added. The precipitate was filtered off and dissolved in water. This water solution was dried on enough dextrin to make one gram of dextrin equivalent to six grams of hog liver and the product was fed wherever G (liver) is indicated in the experimental work.

Care of Animals

Reproduction Studies with Cod Liver Oils.

Vigorous young rats four to five weeks of age and weighing between forty five and fifty five grams were placed in cages 12" x 24" x 10" in groups of six or seven animals. Shavings were used for litter. Three females and three males were used in the groups containing six rats while four females and three males were used in the groups containing seven rats. The animals were weighed every two weeks. Pregnant females were left in the cage with the other rats and the young were removed at birth except in cases where lactation was being studied. When lactation was studied the female was removed to a separate cage for delivery of the young and returned to the original cage after weaning the young.

Diets were mixed daily to avoid any loss of vitamins in the oils studied. Tap water was available at all times from bottles equipped with bent glass tubes.

Lactation Studies with Cod Liver Oils.

Females in advanced pregnancy were removed to separate cages where they remained until the young were weaned at the age of thirty days. After the young were weaned the female was returned to the cage from which she had been taken originally.

Lactation Studies with Grains.

Pregnant females from the stock colony were placed in individual cages and fed the experimental diet from the day of parturition until the young were weaned at twenty eight days of age. Females and litters were kept on wire screens. These two mesh to the inch false screen bottoms were placed over the pans containing shavings. The wide mesh permitted the young to fall through the screens during the first week of life. To avoid this difficulty galvanized iron pans 6" x 8" x 1" were filled with shavings and placed on the screens. These pans served as nests for the young during the first week and were then removed. Food and water were available to the animals at all times

Presentation of Data

1. The Vitamin E Content of Cod Liver Oils.

Eight different brands of cod liver oil were used in this investigation. Vigorous young rats weighing between forty five and fifty five grams were placed on the diet in groups of six or seven. The number of females in each group was never less than three and the number of males was never less than two. The diets contained casein 18%, yeast 12%, salt mixture (185) 3.7%, varying amounts of various cod liver oils and the balance to 100% of dextrin. The oils were mixed into the diets daily to prevent oxidative changes. The number of young served as a measure of the vitamin E content of the oil.

Individual records concerning each female used in this work appear in the supplementary tables XIV to XXVII inclusive. There are two reasons for using different numbers of females on different diets. In some cases when a female died she was replaced by another female of forty five to fifty five grams from our stock colony. In other cases the entire experiment was duplicated. These variations in technique, together with the fact that certain females were allowed to raise their young as a measure of their ability for lactation, may make the results shown in table I a trifle misleading. Exact details regarding each female appear in tables XIV to XXVII inclusive and the reader should consult these tables for exact information.

Resorption of the foeti is characteristic of females fed diets deficient in vitamin E. It seems reasonable to assume that small amounts of the vitamin might enable the female to bring the young nearly to term, or to deliver the young, but to die as a result of the effort. Table I summarizes the information given in tables XIV to XXVII inclusive regarding these points, together with information concerning reproduction.

Table I

Reproduction on diets containing cod liver oil

Diet No.	Name of oil	% oil	Number of females	Number of young	Females died pregnant	Females died in parturition
1	Sea Pure	1	6	0	0	0
2	Sea Pure	2	5	27	1	0
3	Sea Pure	3	10	17	1	1
4	Sea Pure	4	6	7	5	0
5	Sea Pure	5	12	72	4	1
6	Sea Pure	10	5	22	5	0
7	Des Moines Drug	1	5	0	0	0
8	Des Moines Drug	2	5	10	1	0
9	*Des Moines Drug	3	11	78	4	1
10	*Des Moines Drug	4	9	36	2	1
11	Des Moines Drug	5	15	49	3	4
12	Parke Davis	1	4	8	0	0
13	Parke Davis	2	5	34	2	0
14	Parke Davis	3	8	37	2	0
15	*Parke Davis	4	6	123	1	0
16	Parke Davis	5	12	58	5	1
17	Fuller-Morrison	1	4	0	0	0
18	Fuller-Morrison	2	5	7	1	0
19	Fuller-Morrison	3	10	52	2	0
20	Fuller-Morrison	4	4	8	1	0
21	Fuller-Morrison	5	11	70	2	1
22	Eli-Lilly	1	4	11	0	0
23	Eli-Lilly	2	4	6	0	0
24	*Eli-Lilly	3	8	105	2	1
25	Eli-Lilly	4	4	3	0	0
26	Eli-Lilly	5	10	44	2	4
27	Meads	1	3	0	0	0
28	*Meads	2	4	44	1	0
29	Meads	3	8	13	0	1
30	*Meads	4	4	36	2	0
31	Meads	5	9	51	5	0
32	Marden-Wilde	1	3	0	0	0
33	Marden-Wilde	2	3	0	0	0
34	Marden-Wilde	3	7	8	0	0
35	*Marden-Wilde	4	3	0	0	0
36	Marden-Wilde	5	9	7	2	0
37	Squibbs	1	8	12	1	1
38	*Squibbs	2	8	97	1	0
39	*Squibbs	3	12	156	3	2
40	*Squibbs	4	10	185	4	0
41	*Squibbs	5	17	287	2	3
42	Squibbs	10	5	97	0	0

* Denotes that lactation was permitted on these diets.

In order to facilitate comparison of these various oils, the information in table I is further condensed in table II. Table II shows the arrangement of the various oils in the order of their content of vitamin E.

Table II

Summary of reproduction on cod liver oil diets

Name of oil	Number of females	Young born	Females died in pregnancy	Females died at parturition
Squibbs	60	834	11	6
Meads	28	144	8	1
Parke Davis	35	260	10	1
Eli-Lilly	30	169	4	5
Fuller-Morrison	34	137	6	1
Des Moines Drug	45	173	10	6
Sea Pure	44	145	16	2
Marden-Wilde	25	15	2	0

Squibbs cod liver oil heads the list and is in a class by itself. The next class includes Meads and Parke Davis oils which are of approximately the same potency. The Eli-Lilly oil is inferior to the brands mentioned above and superior to those mentioned below. The fourth class includes Fuller-Morrison, Des Moines Drug and Sea Pure oils. The fifth class includes Marden-Wilde cod liver oil. This oil shows the lowest content of vitamin because it permits practically no reproduction. In the light of recent investigations, it is interesting to note that this particular brand of oil was shipped to us in a metal container and that withdrawal of the oil was accompanied by withdrawal of oxides of iron. This may account for an oxidative destruction of the vitamin in the container.

Throughout this investigation it was apparent that the differences between experiments using the different oils and between experiments using different levels of the same oil were often small, while differences between duplicate experiments were often comparatively large. The author sought some figure to make comparisons more obvious. No claim is made as to the statistical validity of the figure chosen. The calculation was made because it proved useful. The fact that the females were on the diet for varying lengths of time was taken into consideration in this calculation as shown in the example below.

The figure selected for comparison was obtained by dividing the total number of young born to all females on a given diet by the number of months these females had been fed this particular diet. The quotient represents the number of young per female per month on the diet. This calculation is illustrated for diet 43. Four females were fed this diet. One female died at the end of four months while the other three females were continued on the diet for five months. The number representing females x months is thus seen to be 5+5+5+4 = 19. The total number of young born to all females on this diet was 6.

$$\text{Young/females x months} = \frac{\text{Total young}}{\text{Females x months}} = \frac{6}{19} = 0.32 = \text{Index.}$$

The figure thus obtained is called the index. Similar calculations gave an index for each diet containing cod liver oil. These are summarized in table III and show that this method groups the oils into the same classes that were suggested in tables I and II.

Table III

Young per female per month on diets containing cod liver oil

Name of oil	% oil	Index	Name of oil	% oil	Index
Squibbs	1	0.24	Fuller-Morrison	1	0.00
Squibbs	2	1.37	Fuller-Morrison	2	0.19
Squibbs	3	1.86	Fuller-Morrison	3	0.83
Squibbs	4	2.43	Fuller-Morrison	4	0.30
Squibbs	5	2.33	Fuller-Morrison	5	0.90
Squibbs	10	2.16			
Meads	1	0.00	Des Moines Drug	1	0.00
Meads	2	2.32	Des Moines Drug	2	0.29
Meads	3	0.43	Des Moines Drug	3	1.15
Meads	4	1.80	Des Moines Drug	4	0.78
Meads	5	1.00	Des Moines Drug	5	0.67
Parke Davis	1	0.28	Sea Pure	1	0.00
Parke Davis	2	1.10	Sea Pure	2	0.82
Parke Davis	3	0.70	Sea Pure	3	0.28
Parke Davis	4	2.27	Sea Pure	4	0.24
Parke Davis	5	0.88	Sea Pure	5	0.91
			Sea Pure	10	1.05
Eli-Lilly	1	0.46	Marden-Wilde	1	0.00
Eli-Lilly	2	0.34	Marden-Wilde	2	0.00
Eli-Lilly	3	2.23	Marden-Wilde	3	0.19
Eli-Lilly	4	0.19	Marden-Wilde	4	0.00
Eli-Lilly	5	1.05	Marden-Wilde	5	0.14

Some investigators have stated that cod liver oil was notably deficient in vitamin E while butter fat contained appreciable quantities of this vitamin. Experiments designed to check the accuracy of this statement are summarized in table IV. Diet 41 is included for comparison. This diet is shown in table I. It contained 5% of Squibbs cod liver oil. Diet 47 is also included for comparison. It is the diet fed to our stock colony. Diets 43 and 44 contained butter fat. The investigation had already shown that 1% of Squibbs cod liver oil was not sufficient for normal reproduction. It might be argued that the deficiency of vitamin A was the reason for this failure. Diet 43 contained 0.5% Squibbs cod liver oil, casein 18%, salt mixture (185) 3.7%, yeast 12%, butter fat 5% and the balance to 100% of dextrin. Diet 44 was identical with diet 43 except that it contained only 0.1% Squibbs cod liver oil. Both diets contained ample amounts of vitamin A.

It seemed wise to study other sources of the vitamin B complex. Diet 45 was identical with diet 41 except that the 12% of yeast in diet 41 was replaced by 12% of extracted wheat embryo. Diet 46 was identical with diet 41 except that the yeast in diet 41 was replaced by 12% of natural wheat embryo in diet 46.

Table IV

Comparisons of reproduction on diets containing cod liver oil and butter fat and on diets containing yeast, wheat embryo or extracted wheat embryo.

Diet No.	Index	% BUTTER fat	Yeast	Ext. wheat embryo	Unext. wheat embryo	Number of females	Months on diet	Young born	% Squibbs oil
41	2.33		12			17	8	287	5
43	0.32	5	12			4	5	6	0.5
44	0.00	5	12			4	5	0	0.1
45	3.82			12		7	10	237	5
46	1.97				12	3	10	59	5
47	4.90					15	12	788	

Results shown in table IV certainly prove that 5% of butter fat is of practically no value from the standpoint of reproduction. These results further show that diet 46 containing natural wheat embryo is superior to diets 43 and 44 containing yeast and butter fat, but inferior to diet 41 containing 5% of Squibbs cod liver oil. Diet 45 containing extracted wheat embryo is decidedly better than diets 41, 43, 44 or 46. Diet 47 was obviously the best of the group. The composition of diet 47 is shown in table V.

Table V

Composition of diet 47

Component	Parts	Weights	% by Volume	% by Weight
Yellow corn	4	43.375	34.8	37.5
Oats groats	4	40.625	34.8	35.2
Alfalfa	1	5.437	8.7	4.7
Wheat	1	10.687	8.7	9.25
Dried butter milk powder	1/2	3.000	4.35	2.6
Tankage	1/2	6.937	4.35	6.0
Linseed oil meal	1/2	5.562	4.35	4.82

In order to determine whether females had become permanently sterile, individual animals from certain groups were fed new diets for a period of three months. Information concerning all these changes of diet is summarized in table XXIV. The results obtained in some of these experiments are mentioned here because they seem especially significant.

Diet 43 contained 5% of butter fat. Four females on this diet had produced one litter of six young in five months. The female giving birth to this litter had died after four months on the diet. The remaining three females were fed diet 47. It has been previously mentioned that diet 47 is used for the maintenance of our stock colony. On this new diet each of the three females produced one litter each in the three months during which the experiment was continued. Diet 44 containing 5% of butter fat failed to permit four females to produce any young in a period of five months. When these four females were fed diet 47 for three months, two of them gave birth to three litters totaling twenty one young. Diet 7 contained 1% of Des Moines Drug cod liver oil. Five females maintained on this diet for ten months failed to give birth to any young. These females were fed diet 47 for three months. During this period three of the five females each gave birth to one litter. The total number of young was twenty nine. Diet 17 contained 1% of Fuller-Morrison cod liver oil. Four females maintained on this diet had failed to give birth to any young in ten months. One female had died at the end of seven months. When diet 47 was fed to the remaining three females for three months, they produced three litters or a total of twenty five young. Diet 35 contained 3% of Meads cod liver oil. Three females were fed this diet for five months and produced no young. During this period two of the females had died. The remaining female was fed diet 42 containing 10% of Squibbs cod liver oil for three months and gave birth to three litters totalling fifteen young. In each case where changes of diet were made, all animals remaining on the experiment were transferred to the new diet. The ability to reproduce shows that both males and females were fertile when fed the new diet.

In reviewing the work concerned with diets 1 to 47 inclusive, the most remarkable finding was the evident superiority of diet 45 which contained extracted wheat embryo. This diet permitted better reproduction than any other diet except the one used for our stock colony. The extracted wheat embryo had been treated with ether for a period ranging from forty eight to sixty hours as described previously. The extracted material contained less than 0.3% of ether soluble material. This same improvement in reproduction was noted by Taylor (1930)(82) in this laboratory when he fed extracted wheat embryo identical with that used by the author.

2. Seasonal Variation in Reproduction and Lactation.

During the progress of the first portion of this research rather marked variations were noted between duplicate experiments carried out during different seasons of the same year and during the same season in different years. In order to check the technique duplicate experiments were performed by different individuals but similar differences were still noted. In order to determine whether the same variations would appear with normal animals on a normal diet, an investigation of reproduction in our stock colony was undertaken. This investigation was complicated by variations in the number of stock animals required to supply the laboratory with experimental animals. A brief description of these requirements follows.

In order to supply the demand for young rats our laboratory maintains a large number of females at all times. The maximum demand for young rats varies but it is usually greatest during October and February. Previous to these periods the number of females bred is considerably increased. Recently a large amount of work in this laboratory involved the use of pregnant females from the stock colony. This introduced a further complication in observing normal reproduction. Two technicians are in general charge of the laboratory and students are hired to care for the animals. To collect the data shown in table VI these students were instructed to place a card on the cages in which they placed females in advanced stages of pregnancy. When the young were born the date of birth and the number of young were written on this card. When the young were weaned the date of weaning and the number weaned were written on this same card. These cards were handed to the author who tabulated the results. During the twenty eight months summarized, the number of females bred varied from two hundred and sixty to five hundred and eighty.

During February of 1930 twenty pregnant females were removed from the stock colony for experiment. During March of 1930 one hundred and fifty pregnant females were removed. In June of 1930 the old females of the colony were replaced by young females. During October of 1930 thirty four pregnant females were removed. During November of 1930 ten more pregnant females were removed. In December of 1930 fifty six additional females were removed for experiment. In April of 1931 the old females of the stock colony were again replaced with young females. These changes in the stock colony certainly altered the apparent fertility of the group. The author knows of no way in which a proper correction can be applied to take care of these changes. It will be noted that the variations in reproduction and lactation bear no close relationship to these changes in the colony and the existence of seasonal variation is still obvious. Table VI is self explanatory and shows that the number of young per female per month varied from 0.22 to 2.06 while the mortality of the young varied from 3.6% to 64.3%. This record was not carried beyond 1932 but during the summer of 1934 the intense heat threatened the destruction of our entire stock colony. The colony was saved by the installation of an air-conditioning system. The system of air-conditioning reduced the temperature of the laboratory but the humidity is high when the fans are sucking the air through a water spray. It is impossible to reduce both temperature and humidity and maintain uniform conditions at the present time.

The results of this investigation go far toward explaining variations in duplicate experiments using various cod liver oils. A continuation of this study over a period of years should prove helpful in the interpretation of the results of experiments.

Table VI

Reproduction and lactation in our stock colony.

Seasonal variation.

Month	Total No. of females	Litters	Avg. size litter	Young born	Young weaned	Mortality	Young per female
			1929				
Dec.	470	66	7.0	465	271	41.7	0.99
			1930				
Jan.	470	104	6.8	711	467	34.5	1.51
Feb.	470	82	6.5	535	389	27.3	1.14
March	320	84	7.5	636	425	33.2	1.99
April	290	47	8.0	375	308	18.0	1.30
May	340	51	8.0	408	316	22.5	1.20
June	260	72	7.4	536	490	8.6	2.06
July	260	40	6.9	277	267	3.6	1.06
August	300	50	6.3	313	290	7.3	1.04
Sept.	580	98	6.9	672	597	11.1	1.16
October	580	59	6.2	366	308	16.0	0.28
Nov.	580	61	6.0	354	273	23.0	0.61
Dec.	440	17	5.8	98	35	64.3	0.22
			1931				
Jan.	500	85	6.45	549	268	51.2	1.10
Feb.	465	75	6.7	503	249	50.0	1.12
March	456	83	6.7	558	248	55.0	1.20
April	460	28	6.5	181	144	20.4	0.34
May	483	20	7.0	141	84	40.4	0.29
June	465	52	6.4	330	172	47.9	0.71
July	465	50	7.1	355	257	27.6	0.72
August	465	34	5.7	195	145	25.6	0.40
Sept.	465	92	6.4	585	444	24.1	1.25
October	465	78	6.13	478	351	26.6	1.02
Nov.	465	91	6.17	562	368	34.0	1.20
Dec.	465	89	6.35	565	335	40.1	1.20
			1932				
Jan.	465	112	6.35	711	363	48.9	1.52
Feb.	465	131	6.42	842	552	34.4	1.81
March	465	75	7.0	524	332	36.6	1.26
April	465	98	6.9	671	535	20.3	1.41

3. Cod Liver Oil Diets for Lactation.

Mothers were permitted to raise their young on certain of the diets containing cod liver oil. All diets used for this purpose have been discussed in connection with table I or table IV. Reproduction on diets 10 to 41 inclusive was summarized in table I while reproduction on diets 45 and 46 was summarized in table IV. It has been pointed out that reproduction was lower when lactation was permitted because the mothers were placed in separate cages where they nursed the young for thirty days.

Lactation studies are summarized in table VII. The mortality and the weaning weights give figures which can be used to judge the success of lactation.

Table VII

Lactation on diets containing cod liver oils

Diet No.	% oil	Number of females	Months on lactation	Total young born	Average weaning wt. Gms.	Total young weaned	% Mortality
*41	5	9	4	159	47	130	18.2
*40	4	6	4	106	45	76	27.3
*39	3	5	4	81	49	64	19.7
*38	2	4	4	63	48	43	31.7
10	4	2	3	28	44	17	40.0
15	4	4	4	55	36	26	52.7
24	3	4	3	35	38	10	71.4
30	4	1	3	19	47	3	84.2
28	2	2	4	18	40	4	77.7
35	4	1	2	15	31	8	47.0
*45	5	7	4	154	44	107	30.5
*46	5	3	4	50	45	17	66.6

* Denotes Squibbs cod liver oil.

Examination of table VII shows conclusively that Squibbs cod liver oil is superior to the other brands studied. This is equally evident if we judge lactation on the basis of the mortality of the young or on the basis of mortality in conjunction with weaning weight. If a specific lactation factor accounts for observed differences in lactation it is obvious that Squibbs cod liver oil contains more of such a factor than other brands studied. Diet 35 contained 4% of Marden-Wilde cod liver oil. At the end of five months on this diet only one female remained alive. The results shown in table VII for diet 35 are those obtained when this female was fed diet 41 containing 5% Squibbs cod liver oil for two months. This shows that Squibbs oil must contain enough vitamin E to permit reproduction in rats which were previously maintained on a diet deficient in this vitamin and further permits the lactation of some of these young after their birth. Table VII shows that diet 45 containing 12% of extracted wheat embryo in place of the 12% of yeast used in other diets is decidedly superior for lactation. It was shown previously that this diet permitted much better reproduction than the diets containing yeast, provided that both diets are supplemented with Squibbs cod liver oil.

All lactation studies were made in conjunction with similar studies wherein the growing ration was used as a control. In every case lactation on the growing ration was better than lactation on the experimental diet. This suggested an investigation to determine how much of the growing ration must be added to synthetic diets to permit satisfactory lactation.

4. The Supplementary Value of the Growing Ration for Reproduction and Lactation.

The composition of the growing ration or diet 47 was shown in table V. The consistent superiority of this diet for reproduction and lactation suggested that small amounts of this mixture of natural foods might supplement synthetic diets in some way which would permit good reproduction and lactation. Results of experiments designed to test this theory are shown in table VIII.

Table VIII

Reproduction and lactation on diets supplemented by the growing ration.

Diet No.	% of diet 47 added	Number of females	Months on diet	Young born	Young weaned	Average weaning wt. Gms.	% Mortality
50	2	3	12	0	0		
51	5	3	15	0	0		
52	10	3	15	0	0		
53	15	3	15	21	0		100.0
54	20	3	15	88	3	28	96.0
55	25	3	15	88	19	30	76.0
57	50	3	15	91	55	45	40.0
58	73	3	15	76	71	44	6.6
59	95	6	15	207	171	47	17.4
60	100	14	10	358	281	41	21.5
61	95	3	10	112	86	41	23.0
62	95	2	10	37	30	55	19.0
63	100	3	10	104	64	53	38.0
64	100	3	10	80	64	51	20.0

Diets 50 to 58 inclusive all contained casein 18%, salt mixture (185) 3.7%, butter fat 5% and different amounts of diet 47 with the balance to 100% made up with dextrin. The amounts of diet 47 are shown in column two of table VIII. Diets 59, 61, 62, 63 and 64 were slight modifications of the original diet 47. Diet 59 contained 5% of butter fat. Diet 61 contained 5% of Squibbs cod liver oil. Diet 62 contained 5% of fish meal. Diet 63 was supplemented by fish meal ad. lib. The fish meal was fed in a separate feed cup. The tankage in diet 47 was replaced by an equal amount of fish meal to make diet 64.

Three females and three males, weighing between forty five and fifty five grams, were placed in each group and maintained on the diet for five months. Females in advanced stages of pregnancy were removed to separate cages and allowed to raise their young. After weaning the young, the female was returned to the cage containing the other rats of her original group.

The results shown in table VIII reveal that success in lactation varies directly with the amount of the growing ration included in the diet. Butter fat improves the growing ration. Fish meal contributes some factor which permits the rearing of larger young.

5. Lactation on Diets wherein Individual Components of the Growing Ration Supplement Wheat.

Previous work in this laboratory (21) had shown that purified diets supplemented with 40% of wheat had failed to permit proper lactation. In this previous work young animals had been placed on the selected diet at the time of weaning and maintained on that diet until they died. Purified diets supplemented by 40% wheat failed to permit proper lactation. Since our growing ration allowed proper lactation the question arose as to which particular component of this ration was responsible for proper lactation. The most rapid method available for obtaining this information was the use of pregnant females from our stock colony. This was the method used. Six females were fed the selected diet from the time of parturition to the time of the weaning of the young. Experiments using diets 75 and 77 were duplicated. Litters were reduced to six if more than this number of young were born. The diets used in this work all contained casein 18%, butter fat 5%, salt mixture (185) 3.7%, wheat 40%, varying amounts of various component of the growing ration and the balance to 100% of dextrin.

Table IX

Composition of diets supplementing wheat

Diet number	Component supplementing wheat	% of Component
75	None	0
76	Butter milk powder	10
77	Butter milk powder	20
78	Linseed oil meal	10
79	Linseed oil meal	20
80	Tankage	10
81	Tankage	20
82	Fish meal	10
83	Alfalfa	20

Six females were placed on each of the above diets at parturition and were allowed to nurse the young until the twenty eighth day. The results of the investigation are shown in table X. The mortality and the weaning weights serve as measures of success in lactation.

Table X

Lactation on diets supplementing wheat

Diet No.	Number of females	Young born	Young weaned	% mortality	Average weaning weight at 28 days
75	12	70	43	38.5	36.0
76	6	34	32	6.0	48.9
77	11	67	64	4.5	61.3
78	6	36	33	8.3	36.1
79	6	35	33	5.7	35.7
80	6	35	18	48.6	30.2
81	6	35	11	70.0	48.9
82	6	35	35	0.0	43.5
83	6	34	32	6.0	39.5

It was stated above that experiments using diets 75 and 77 were duplicated. This accounts for the number of females being increased on these two diets.

The summary in table X shows that tankage is detrimental rather than beneficial as a supplement to the wheat diet. All other added components improve the wheat diet for lactation. The author attaches no significance to variations in mortality of less than 10%. Individual differences in the animals may account for larger variations in mortality. It is significant when six different females are able to wean all their young on a given diet. The mortality was 0.0% on diet 82 which contained 10% of fish meal. Diet 77 contained 20% of butter milk powder and females on this diet were able to wean especially fine young which weighed considerably more than normal young at this age. Mothers on diets containing tankage, diets 80 and 81, ate very little of the diets and sometimes ate the young. Extreme nervousness was characteristic of mothers and young and the young were often in a state of paralysis preceding death. The high mortality on diet 81 undoubtedly accounts for the high weaning weight, since almost two thirds of the young died well before the date of weaning. A similar nervousness developed in animals on diet 75 in which wheat was the sole source of vitamins B and G. Paralysis preceding death was often noted in young of mothers on this diet.

6. Individual Components of the Growing Ration as Sources of Vitamins B and G for lactation.

The work reported above showed that lactation on purified diets was improved when such diets were supplemented with wheat plus various components of the growing ration. It seemed logical to investigate each of these components as the sole supplementary source of vitamins B and G. It was still a question as to whether wheat might contain one factor for lactation while the supplementary components of the growing ration contained a different factor, or whether the same essential factor was supplied in part by each of these supplements of the purified diets. A series of experiments was planned to answer this question. Diets containing different percentages of each component were fed to females from our stock colony from the day of parturition until the young were weaned. These diets contained casein 18%, Squibbs cod liver oil 4%, salt mixture (185) 3.7%, varying percentages of the added components and the balance to 100% of dextrin. The levels selected were 10%, 25%, 40% and 60% for each component used. Six females were used on each diet and litters containing more than six young were reduced to six. The cod liver oil was mixed into the diet daily to prevent any oxidative destruction of the vitamins. The results of this investigation are shown in table XI. A more detailed summary appears in table XXVII.

Table XI

Mortality after the first week on diets containing individual components of the growing ration as sources of vitamin B and of vitamin G.

Component used as sole source of vitamins B and G	% mortality on diets with amounts of added component shown below.			
	10%	25%	40%	60%
Wheat	64	69	65	39
Alfalfa	71	23	42	30
Tankage	100	100	100	100
Butter milk powder	83	88	100	74
Fish meal	100	82	91	80
Linseed oil meal	76	41	86	100
Cotton seed meal	83	50	80	83

The results shown in table XI show that all of these diets were unsatisfactory for lactation. The mixture of wheat with the other components must account for the low mortality shown in table X. In order to determine whether vitamin B or vitamin G, or the combination of these two vitamins, would satisfactorily supplement these various components of the growing ration, another series of experiments was planned. Diets containing 25% of the growing ration component, casein 18%, Squibbs cod liver oil 4%, salt mixture (185) 3.7% and the balance to 100% of dextrin were supplemented as shown below. Vitamin B was supplied by feeding 0.3 grams of the vitamin B concentrate previously described to each mother daily. Vitamin G was supplied by feeding each female 1.5 grams of the vitamin G concentrate prepared from liver as previously described. This supplement was fed daily. The combination of both vitamins was supplied by feeding the above amounts of each preparation to the female. In order to compare the vitamin G concentrate with the vitamin in autoclaved yeast another group of females was supplied daily with 0.3 grams of the vitamin B concentrate plus 2.4 grams of the autoclaved yeast. The reason for selecting these amounts of the supplements was that Feaster (18) had shown that normal lactation required six times the amount of each vitamin needed for normal growth.

Detailed results of this investigation appear in table XXVII. Mortalities in this table as well as mortalities shown in table XII are calculated on the basis of the number of young living at the end of the first week. It seemed reasonable that the diet could scarcely be responsible for loss of young during the first week because the mother had been on the stock diet preceding parturition. Results are more reasonable if this figure is used although diets containing alfalfa and cotton seed meal show a better comparison if total mortalities are used. The figure for 25% alfalfa with no vitamin supplement is 23% mortality after the first week and 36% total mortality. For cotton seed meal mortality after the first week is 50% while the total mortality is 84%.

Comparative mortalities on diets containing 25% of the various components of the growing ration appear in table XII. Mortalities on these diets supplemented with vitamins B and G are also shown.

Table XII

Mortality after the first week on diets containing individual components of the growing ration, with and without supplements of vitamins B and G. (%).

Component of the growing ration	No vitamin	Vitamin B	Vitamin G (liver)	Vitamins B and G (liver)	Vitamins B and G (yeast)
Wheat	69	32	20	0	3
Alfalfa	23	39	33	0	18
Tankage	100	31	72	11	9
Butter milk powder	88	17	44	3	3
Fish meal	82	58	100	0	0
Linseed oil meal	41	20	0	0	9
Cotton seed meal	50	0	12	0	6
Growing ration	20	0	12	3	11

Table XII shows that all diets gave lower mortality figures when supplemented with vitamin B or vitamin G with the exception of the diet containing alfalfa. The alfalfa diet showed no improvement with either vitamin. All diets except that containing alfalfa showed improvement with vitamin B and all except those containing alfalfa or fish meal showed improvement with the addition of vitamin G. This would indicate that alfalfa contained enough of both vitamins and that fish meal contained enough vitamin G. A greater fall in mortality resulted from the addition of vitamin B than from addition of vitamin G in the cases of tankage, butter milk powder, fish meal and cotton seed meal indicating that these components are deficient in vitamin B. In like manner, a greater fall in mortality resulted from the addition of vitamin G than from the addition of vitamin B in the cases of wheat and linseed oil meal indicating that these components are deficient in vitamin G. The addition of both vitamins reduced mortality more than either vitamin alone in the cases of wheat, alfalfa, tankage, butter milk powder and fish meal. This would indicate a deficiency of both vitamins. It is interesting to note that the addition of both vitamins permits lactation equivalent to the lactation on our stock ration in every case studied.

The figures shown in table XII form the basis for the conclusions in the preceeding paragraph. The author feels that some of these conclusions may not be justified. Experience has shown that differences in mortality of 12% are hardly significant. Duplicate experiments would reduce these differences and might conceivably reverse the apparent superiority of one diet over the other. If duplicate experiments showed the same relationships repeatedly, the conclusions would be justified. If we now modify the conclusions of the preceding paragraph with this in mind we would conclude that vitamin B supplements the diets better than vitamin G in the cases of tankage, butter milk powder and fish meal, while vitamin G supplements the diet better than vitamin B in the case of linseed oil meal.

The vitamin G preparation made from liver seems to give better results than the autoclaved yeast in diets containing wheat, alfalfa, linseed oil meal, cotton seed meal and even with the growing ration itself. These differences are slight in every case except that of the alfalfa diet. Unfortunately, the work with alfalfa failed to give consistent results throughout this investigation. Duplicate experiments failed to correct these inconsistencies. The author believes that alfalfa is unsatisfactory when used in diets for rats. This would modify all conclusions involving alfalfa diets and would indicate caution in assuming a decidedly superior supplementary value for the vitamin G prepared from liver.

The preceding discussion concerned the mortality of the young on the various diets. A study of the weights of the young at the time of weaning is equally instructive. The young were weaned at twenty eight days. It is not safe to consider weights of the young as the sole measure of improvement in lactation. High mortality may accompany high weaning weight since the mother has fewer young to suckle. Complete records of all this work appear in table XXVII while the comparative weaning weights are further summarized in table XIII.

Table XIII

Weaning weights of young on diets containing the components of the growing ration with and without vitamin B or G supplements. (Grams).

Component of the growing ration	Per cent of the added component 10%	25%	40%	60%	Vitamin supplements B	G	B+G liver	B+G yeast
Wheat	15	19	21	26	21	48	58	41
Alfalfa	19	24	18	15	22	39	46	28
Tankage					12	16	32	35
Butter milk powder	19	25		33	31	27	53	49
Fish meal		26	17	22	33		57	46
Linseed oil meal	17	21	21		24	35	49	50
Cotton seed meal	22	30	16	18	32	55	53	43
Growing ration 100% + vitamin supplement					48	49	49	53
Synthetic diet + vitamin supplements as shown							45	45

The information shown in table XIII suggests that vitamin B supplements diets containing tankage, butter milk powder and fish meal. Vitamin G appears to supplement diets containing wheat, alfalfa, tankage, linseed oil meal and cotton seed meal. The supplementary value of B is greater than that of vitamin G in the case of fish meal. The supplementary value of vitamin G is greater than that of vitamin B in diets containing wheat, alfalfa, linseed oil meal and cotton seed meal. The addition of both vitamins improves all diets more than either vitamin alone with the exception of those containing cotton seed meal. Cotton seed meal is supplemented as adequately with vitamin G as with both vitamins. A study of weaning weights indicates that fish meal, butter milk powder and tankage are deficient in vitamin B while wheat, alfalfa, tankage, linseed oil meal and cotton seed meal are deficient in vitamin G.

It is obvious that conclusions in regard to deficiencies in these diets differ when we consider mortalities in one case and weaning weights in the other case. Combining the evidence from both angles we see that deficiency of vitamin B exists in diets containing tankage, butter milk powder, fish meal, linseed oil meal and possibly cotton seed meal. Deficiency of vitamin G exists in diets containing wheat, tankage, butter milk powder, linseed oil meal and cotton seed meal. It further appears that our growing ration may be deficient in both vitamins B and G.

It is entirely possible that these conclusions may be in error if some factor essential for satisfactory lactation was present in some of these diets. The existence of such a lactation factor would alter all conclusions regarding the value of the other vitamins in the diet.

DISCUSSION OF RESULTS

This research proved that the vitamin E content of different brands of cod liver oil varied widely. Squibbs oil was outstandingly better than other brands studied. Meads oil contained appreciable amounts of vitamin E while Sea Pure oil contained very little of this vitamin. These results are in accord with previous work of Nelson and co-workers (6, 50, 53, 85) who have reported consistent reproduction on diets containing Squibbs oil, and with the work of McCollum and co-workers (64) who reported the probable presence of vitamin E in Meads oil. The results are partially in accord with those of Sure (76) who reported that Sea Pure oil was free from vitamin E. The present research revealed such a small content of vitamin E in Sea Pure oil that it might be considered practically free of vitamin E. Results of this research are partially in agreement with the statement made by Evans (15) who used Meads oil in certain experiments and reported that cod liver oil was notably deficient in vitamin E, provided we consider the subsequent statement by Evans (15) that lard contains some substance which destroys vitamin E in cod liver oil when both are present in the diet. Mattill (36, 37) offerred an explanation of the divergent reports of Nelson and Evans which seemed reasonable and sufficient. Nelson (5, 53) explained this difference in results in much the same manner. Oxidative destruction of vitamins A and E and variations in the original content of different oils in these vitamins account for the divergent results in different laboratories.

Experiments using different cod liver oils in lactation studies showed that Squibbs oil was superior to all other brands for lactation. There might be two explanations for this superiority. Evans (15) reported that vitamin E is essential for proper lactation. Sure (74) reported that certain foods rich in vitamin E also contained a lactation factor. The existence of such a lactation factor has been postulated by numerous investigators. A higher content of vitamin E or an appreciable content of such a lactation factor would account for the superiority of Squibbs oil in promoting lactation.

This research demonstrated the superiority of Squibbs oil as compared with butter fat in promoting reproduction and lactation. This is in agreement with previous work of Nelson and co-workers (44, 18). Reports from other laboratories using different brands of cod liver oil and different percentages of butter fat are hardly comparable.

The use of extracted wheat embryo as the source of vitamins B and G in diets containing Squibbs oil gave consistently better results than the use of natural wheat embryo or yeast when such diets were used to study reproduction and lactation. This is in agreement with previous work by Nelson and co-workers (4, 44, 82). An explanation might be sought in studying extracted wheat embryo to determine its content of traces of vitamin E or its content of anti-oxidants.

Studies of reproduction and lactation in the stock colony revealed variations in normal animals on a normal diet which far exceeded the differences previously deemed significant in experimental animals. This study can scarcely be compared with results reported by other investigators unless detailed descriptions of the control of laboratory conditions should accompany such reports. There is fairly general agreement in the literature that control of temperature, humidity, light and sanitation is absolutely essential. Investigators who report no seasonal variations are those whose laboratories are carefully controlled in regard to these factors. Workers who report seasonal variation fail to discuss these factors in sufficient detail to make a critical study of the problem. The author believes that the first step in any quantitative or accurate study employing experimental animals should be preceded by well planned and exhaustive studies of variations within the colony. Curves showing these variations should be compared with records of temperature, humidity and sanitation and the limits of normal variation should be established before any comparative studies could be reported as significant.

Varying amounts of our growing ration have been shown to supplement diets containing no other provision for vitamins B and G. The supplementary value of the added growing ration was progressively greater as the percentage was increased in these diets. This indicated that our growing ration may be deficient in vitamins B and G. The growing ration itself is improved by the addition of butter fat which indicates that it may be deficient in vitamin A. Fish meal was shown to supplement the growing ration. This might be explained on the basis of added vitamin G or on the basis of an improved protein supply.

Diets containing 40% of wheat have been shown to be unsatisfactory for lactation. This is in agreement with Nelson and co-workers (18, 21, 82, 85) and with Aykroyd and Roscoe (2) but is contradictory to the report of Hunt (26) who found that 35% of wheat permitted normal lactation. There is no real basis for comparisons of lactation on wheat diets unless a complete description of the wheat is available. Aykroyd and Roscoe (2) reported wide variations in the vitamin B content of wheat from different sources. Lactation on wheat diets, in the present research, was improved by the addition of butter milk powder, linseed oil meal, fish meal or alfalfa. Lactation on diets in which tankage supplemented the wheat diet was poorer than that on the wheat diet itself. Butter milk powder supplemented the wheat diet most successfully while fish meal was a very good supplement. These results indicate that the deficiency in the wheat diet is one of vitamin G. This is in agreement with the work of Sherman (61), Feaster (18) and Wilkinson (85).

Diets adequate in all the dietary requirements except vitamins B and G were supplemented with various components of the growing ration to determine their value in lactation. These components were fed at different levels up to 60% of the diet. Lactation was below normal in every case showing that no one of these components furnished enough of these vitamins when fed in amounts equal to 60% of the diet. This is in agreement with many workers who have reported that the lactating mother requires considerably more of these vitamins than the growing animal.

Lactation on diets containing 25% of the various components of the growing ration was compared with lactation on diets in which these components were supplemented with vitamins B and G. This revealed certain vitamin deficiencies for normal lactation. Vitamin B appeared to be the limiting factor in tankage, butter milk powder, linseed oil meal and fish meal, while vitamin G appeared to be the limiting factor in wheat, tankage, butter milk powder, linseed oil meal and cotton seed meal. Results with wheat are in agreement with Sherman and Axtmayer (6) who reported that vitamin G was the limiting factor in wheat. Results with alfalfa are not in accord with Osborne and Mendel (57) who reported that alfalfa was a good source of vitamins B and G. The author used much larger amounts of alfalfa than these investigators and this probably accounts for the result. Results with cotton seed meal indicate that it was deficient in vitamin G but furnished almost enough vitamin B for lactation. The literature concerning vitamins B and G in cotton seed meal relates to its value for growth. Hunt (27) reported that cotton seed meal was a good source of vitamin B. Munsell (46) found that it was a good source of vitamin B but was deficient in G. Whitsitt (84) reported that cotton seed meal was a rich source of vitamin B and a good source of vitamin G.

The literature revealed no lactation studies of diets in which vitamins B and G were furnished by tankage, butter milk powder, linseed oil meal or fish meal. The author would conclude that 25% levels of tankage, butter milk powder and linseed oil meal failed to provide sufficient vitamin B or vitamin G for satisfactory lactation. Fish meal at this level failed to provide sufficient vitamin B for lactation and cotton seed meal failed to provide sufficient vitamin G for lactation. This research shows that diets containing fish meal are superior to diets containing tankage when such diets are used in lactation studies.

All the conclusions drawn from the results of this research must be modified if a specific factor for lactation is shown to be present in the foods studied. This is a possibility which should be considered in all work involving lactation studies.

SUMMARY AND CONCLUSIONS

Reproduction was used as a measure of the vitamin E content of eight different brands of cod liver oil. Diets tested contained varying amounts of these different oils which furnished vitamins A, D and E in such diets. A comparison of the number of young born to females on these diets showed that the oils could be arranged in five groups according to their content of vitamin E. Squibbs oil was markedly superior to the other oils studied and it constituted group one. Meads and Parke Davis oils seemed of about equal potency and constituted group two. Eli-Lilly oil was inferior to the oils mentioned above and constituted group three. Fuller-Morrison, Des Moines Drug and Sea Pure oils were of approximately the same value and together constituted group four. Marden-Wilde oil constituted group five. It was inferior to all other oils studied and permitted the least reproduction. Diets containing butter fat as the sole source of vitamins A, D and E were inferior to those containing an equal percentage of Squibbs cod liver oil. This showed that the oil was superior to butter fat for reproduction.

Diets containing extracted wheat embryo as the sole source of vitamins B and G together with 5% of Squibbs cod liver oil as a source of the other vitamins were markedly superior to diets in which yeast replaced the extracted wheat embryo as a source of vitamins B and G. The wheat embryo diet permitted better reproduction and lactation than the yeast diet.

Records of reproduction and lactation in our stock colony showed that there was a large variation using normal animals on a normal diet. This study revealed much larger differences in the stock colony than the differences which had been assumed to be significant in the work involving purified diets.

Lactation studies using various brands of cod liver oil to furnish vitamins A, D and E in the diet showed that Squibbs cod liver oil was superior to all other oils studied. This superiority was shown by lower mortality and higher weaning weights of the young.

Diets consisting of purified foods, adequate in all respects except in vitamins B and G, were supplemented with the growing ration as a source of these vitamins. Studies of lactation and reproduction on these diets showed that 10% of the growing ration was not sufficient to permit reproduction and that increases up to 73% of the growing ration improved reproduction and lactation. The improvement in weaning weights on the diet containing 73% of the growing ration more than offset the slight drop in the number of young born. The addition of butter fat improved the growing ration itself. When the growing ration was supplemented with fish meal the young showed a higher weaning weight and appeared to be better animals than those fed the growing ration without fish meal.

Diets consisting of purified foods and supplemented by 40% of wheat were further supplemented by adding various components of the growing ration. The components used were tankage, butter milk powder, linseed oil meal, fish meal and alfalfa. In every case except the one where tankage was used, lactation was improved by the inclusion of 10% or 20% of these added components. The diet including tankage was poorer for lactation than the diet of purified foods and wheat. The latter diet, containing wheat and no additional supplement, permitted reproduction but many of the young showed extreme nervousness which often developed into paralysis preceding death. This nervousness was much more marked in young on diets containing tankage. The diet containing 20% of tankage was so poor that even the mothers became nervous. Practically all the young on this diet were extremely irritable and the high mortality shows that many died during the lactation period. Butter milk powder was the most satisfactory supplement when fed at a level equal to 20% of the diet. The weaning weights of the young were uniformly higher and the young were especially fine in appearance. The diet containing 10% of butter milk powder was good, and the young were better than the average rats at this age. The diet containing 10% of fish meal was remarkable in that the mortality on this diet was zero.

Diets adequate in all respects except in vitamins B and G were supplemented with various components of the growing ration. Each component was included in four different diets wherein it constituted 10%, 25%, 40%, or 60% of the diet. Lactation was poor on all these diets. In order to determine whether the failure of lactation was due to a lack of vitamins B or G or a lack of both the vitamins B and G, diets were made up containing 25% of the component being studied. These diets were supplemented in four different ways. The first supplement was a vitamin B adsorbate on fuller's earth. The second supplement was a concentrate of vitamin G prepared from hog liver and dried on dextrin. The third supplement consisted of the vitamin B concentrate and the vitamin G preparation from liver. The fourth supplement consisted of the vitamin B concentrate and vitamin G furnished by autoclaved yeast. These diets were fed with and without supplements and comparisons of mortalities and weaning weights were used to indicate vitamin deficiencies in the various components used.

Comparative mortalities indicated that tankage, butter milk powder, fish meal and cotton seed meal were deficient in vitamin B while wheat and linseed oil meal were deficient in vitamin G. Comparative weights of the young at the time of weaning indicated that fish meal, butter milk powder and tankage were deficient in vitamin B while wheat, alfalfa, tankage, linseed oil meal and cotton seed meal were deficient in vitamin G. Deficiencies were evident when both these criteria were used. The final conclusion was drawn that tankage, butter milk powder, fish meal, linseed oil meal and possibly cotton seed meal were deficient in vitamin B while wheat, tankage, butter milk powder, linseed oil meal and cotton seed meal were deficient in vitamin G. The growing ration used for our stock colony was deficient in both vitamins. The addition of both vitamins to any of the diets studied permitted lactation equivalent to the lactation on our growing ration.

The vitamin G preparation made from hog liver appeared to be a more satisfactory supplement than the vitamin G present in autoclaved yeast. Lactation was more satisfactory on diets supplemented by vitamin B plus the liver preparation than on diets supplemented by vitamin B plus autoclaved yeast.

Conclusions regarding the supplementary value of vitamins B or G for any of these diets must be qualified if any of the foods contained some specific lactation factor which is unknown at present. The existence of such a factor has been suggested and is a possibility to be regarded seriously. If these diets contained such a factor, the author was not aware of the fact.

Quantitative studies involving differences as small as those observed in this work should be preceded by a thorough examination of the stock colony. Curves showing the laboratory normal and the usual variations from this normal should be prepared in order to determine whether experimental results are significant.

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LITERATURE CITED

1. Adair, F. L.
The influence of diet on lactation. Am. J. Obst. and Gynec. 9: 1-16. 1925.

2. Aykroyd, W. R. and Roscoe, M. H.
LV. The distribution of vitamin B_2 in certain foods. Biochem. J. 23: 483-497. 1929.

3. Anderegg, L. T.
Diet in relation to reproduction and rearing of young. J. Biol. Chem. 59: 587-599. 1924.

4. Anderegg, L. T. and Nelson, V. E.
Whole and skimmed milk powders as food. Observations on a new vitamin for reproduction. Ind. Eng. Chem. 17: 451-455. 1925.

5. Anderegg, L. T. and Nelson, V. E.
Milk powders as food. II. Observations on the existence of vitamin E. Ind. Eng. Chem. 18: 620-622. 1926.

6. Bills, C. E., Honeywell, E. M., Wirick, A. M. and Nussmeier, Mildred.
A critique of the line test for vitamin D. J. Biol. Chem. 90: 619-636. 1931.

7. Braman, W. W.
Relative values of the proteins of linseed and cotton seed meals in the nutrition of growing rats. J. Nutrition 4: 249-259. 1931.

8. Cummings, M. J. and Mattill, H. A.
The auto-oxidation of fats with reference to their destructive effect on vitamin E. J. Nutrition 3: 421-432. 1930.

9. Daniel, E. P. and McCollum, E. V.
Studies on the nutritive value of fish meals. U. S. Dept. Commerce, Bur. Fisheries. Investigational Rept. 2: 1-19. 1931.

10. Donaldson, H. H.
The Rat. 2d ed. Mem. Wistar Inst. Anat. Biol. No. 6 Philadelphia. 1924.

11. Douglass, Earl.
Deterioration of alfalfa hays resulting from rain.
Colo. Agr. Expt. Sta. 45th Annual Rept. 32-34. 1932.

12. Douglass, Earl, Tobiska, J. W. and Vail, C. E.
Studies on changes in vitamin content of alfalfa hay.
Colo. Agr. Expt. Sta. Tech. Bull. 4: 1-68. 1933.

13. Evans, H. M.
Relation of vitamin E to growth and vigor.
J. Nutrition 1: 23-28. 1928.

14. Evans, H. M.
Vitamin E. J. Am. Med. Assoc. 99: 469-475. 1932.

15. Evans, H. M. and Burr, G. O.
The anti-sterility vitamine fat soluble E.
Mem. Univ. Calif. 8: 1-176. 1927.

16. Evans, H. M. and Burr, G. O.
On the amount of vitamin B required during lactation.
J. Biol. Chem. 76: 263-272. 1928.

17. Evans, H. M. and Burr, G. O.
Development of paralysis in the suckling young of mothers deprived of vitamin E. J. Biol. Chem. 76: 273-297. 1928.

18. Feaster, J. F.
Studies on vitamins B and G in growth and lactation in the rat. (a) The effects of extracts of vitamins B and G. (b) The distribution of vitamin G.
Unpublished thesis. Library. Iowa State College, Ames, Iowa. 1934.

19. Gallup, W. D. and Reder, Ruth.
Calcium salts in the detoxication of gossypol.
Proc. Oklahoma Acad. Sci. 14: 74-75. 1934.
Original not seen. Abstracted in Chemical Abstracts 28: 6796. 1934.

20. Greenbank, G. R. and Holm, G. E.
Some factors concerned in the autoxidation of fats with especial reference to butter fat. Ind. Eng. Chem. 16: 598-601. 1924.

21. Guest, A. E., Nelson, V. E., Parks, T. B. and Fulmer, E. I.
Diet in relation to reproduction and rearing of young.
II. Am. J. Physiol. 76: 339-348. 1926.

22. Hanson, F. B. and Heys, Florence.
Differences in the growth curves of albino rats born during the four seasons of the year under uniform laboratory conditions. Anat. Rec. 35: 83-89. 1927.

23. Hanson, F. B. and Sholes, F. N.
Seasonal differences in sex ratio, litter size and birth weight of the albino rat under uniform laboratory conditions. Genetics 9: 363-367. 1924.

24. Hogan, A. G. and Harshaw, H. M.
Nutritional requirements for normal reproduction. J. Metab. Res. 5: 111-128. 1924.

25. Hogan, A. G. and Harshaw, H. M.
Some relations between fertility and the composition of the diet. Missouri Agr. Expt. Sta. Res. Bull. 94: 1926.

26. Hunt, C. H.
The complex nature of vitamin B as found in wheat and corn. J. Biol. Chem. 78: 83-90. 1928.

27. Hunt, C. H.
Cotton seed meal studies. Ohio Agr. Expt. Sta. Bimonthly Bull. 158: 178-182. 1932.

28. Hunt, C. H. and Krauss, W. E.
The influence of the ration of the cow upon the vitamin B and vitamin G content of milk. J. Biol. Chem. 92: 631- 638. 1931.

29. Hussemann, D. L. and Hetler, R. A.
The vitamin B and G requirements of lactation. J. Nutrition 4: 127-140. 1931.

30. Kennedy, W. P.
Reproduction and diet in the rat. Quart. J. Exptl. Physiol. 16: 281-289. 1926.

31. King, H. D.
Litter production and the sex ratio in various strains of rats. Anat. Rec. 27: 337-366. 1924.

32. King, H. D. and Stotsenburg, J. M.
On the normal sex ratio and the size of the litter in the albino rat (Mus norvegicus albinus). Anat. Rec. 9: 403-420. 1915.

33. Lewis, R. C.
Other factors than vitamins B and G in the vitamin B complex. J. Nutrition 6: 559-570. 1933.

34. McCollum, E. V. and Simmonds, Nina.
The newer knowledge of nutrition. 4d ed.
The Macmillan Company. New York. 1929.

35. McCollum, E. V. and Simmonds, Nina.
A study of the dietary essential, water soluble B, in relation to its solubility and stability towards reagents. J. Biol. Chem. 33: 55-89. 1918.

36. Macy, I. G., Outhouse, Julia, Long, M. L. and Graham, Alice.
Human milk studies. I. Technique employed in vitamin studies. J. Biol. Chem. 73: 153-174. 1927.

37. Mattill, H. A.
The oxidative destruction of vitamins A and E and the protective action of certain vegetable oils.
J. Am. Med. Assoc. 89: 1505-1508. 1927.

38. Mattill, H. A., Carman, J. S. and Clayton, M. M.
The nutritive properties of milk. III. The effectiveness of the X substance in preventing sterility in rats on milk rations high in fat. J. Biol. Chem. 61: 729-740. 1924.

39. Mattill, H. A. and Clayton, M. M.
Vitamin E and reproduction on synthetic and milk diets.
J. Biol. Chem. 68: 665-685. 1926.

40. Mattill, H. A. and Stone, N. C.
The nutritive properties of milk with special reference to reproduction in the albino rat. II.
J. Biol. Chem. 55: 443-455. 1923.

41. Mendel, L. B. and others.
The vitamins. A symposium. American Medical Association. Reprinted for Mead Johnson and company from J. Am. Med. Assoc. 1932.

42. Mitchell, H. H.
On the identity of the water-soluble growth-promoting vitamine and the antineuritic vitamine. J. Biol. Chem. 40: 399-413. 1919.

43. Moore, C. U., Plymate, H. B. and Andrew, B. J.
Studies in the B vitamins. III. Evidence of a third vitamin B factor in yeast (B_4) as shown by growth curves and clinical symptoms of first and second litter young of mothers raised on synthetic B_1 and B_2 diets. Am. J. Physiol. 102: 581-592. 1932.

44. Moore, P. A.
Dietary factors concerned with reproduction. Unpublished thesis. Library, Iowa State College, Ames, Iowa. 1926.

45. Morelle, J.
Influence de la privation de la vitamine E chez le rat au cours de la lactation. Compt. rend. soc. biol. 108: 804-805. 1931.

46. Munsell, H. E. and De Vaney, G. M.
The vitamin B and G content of wheat germ, rice polishings, cotton seed flour, and the residue from fermented rye grains. Cereal Chem. 10: 287-297. 1933.

47. Nakahara, Waro and Inukai, Fumito.
Studies on dietary requirements for lactation. I. Failure of lactation on an apparently complete synthetic diet. Sci. Papers Inst. Phys. Chem. Research (Tokyo). 22: 301-307. 1933.

48. Nakahara, Waro and Inukai, Fumito.
Studies on dietary requirements for lactation. II. Presence of lactation promoting substance in liver extract. Sci. Papers. Inst. Phys. Chem. Research (Tokyo). 24: 33-39. 1934.

49. Nakahara, Waro, Inukai, Fumito and Kato, Shu.
Studies on dietary requirements for lactation. III. The existence of a specific factor for lactation. Sci. Papers Inst. Phys. Chem. Research (Tokyo). 24: 155-161. 1934.

50. Nelson, V. E., Heller, V. G. and Fulmer, E. I.
Studies on yeast. VII. The dietary properties of yeast. J. Biol. Chem. 57: 415-424. 1923.

51. Nelson, V. E., Heller, V. G. and Fulmer, E. I.
Molasses as a source of vitamin B. Ind. Eng. Chem. 17: 199-201. 1925.

52. Nelson, V. E., Jones, R. L., Adams, Georgian and Anderegg, L. T.
Cod liver oil as food. Observations on the existence of vitamin E. Ind. Eng. Chem. 19: 840-844. 1927.

53. Nelson, V. E., Jones, R. L., Heller, V. G., Parks, T. B., and Fulmer, E. I.
Diet in relation to reproduction and rearing of young. I. Observations on the existence of vitamin E. Am. J. Physiol. 76: 325-338. 1926.

54. Nelson, V. E., Ohrbeck, Esther, Jones, R. L. and Taylor, M. W.
Cod liver oil for reproduction. Am. J. Physiol. 85: 476-481. 1928.

55. Olcott, H. S., and Mattill, H. A.
The unsaponifiable lipids of lettuce. III. Antioxidant. J. Biol. Chem. 93: 65-70. 1931.

56. Orent, E. R. and McCollum, E. V.
Effects of deprivation of manganese in the rat. J. Biol. Chem. 92: 651-678. 1931.

57. Osborne, T. B. and Mendel, L. B.
Nutritive factors in plant tissues. III. Further observation on the distribution of water-soluble vitamine. J. Biol. Chem. 41: 451-468. 1920.

58. Reader, Vera.
XI. Further evidence for a third accessary "B" factor. Biochem. J. 24: 77-80. 1930.

59. Reader, Vera.
CCVII. The assay of vitamin B_4. Biochem. J. 24: 1827-1831. 1930.

60. Richardson, A. E. and Green, H. S.
Nutrition investigations upon cotton seed meal. I. J. Biol. Chem. 25: 307-318. 1916.

61. Sherman, H. C. and Axtmayer, J. H.
A quantitative study of the problem of the multiple nature of vitamin B. J. Biol. Chem. 75: 207-212. 1927.

62. Sherman, H. C. and Ellis, L. N.
Necessary versus optimal intake of vitamin G (B_2). J. Biol. Chem. 104: 91-97. 1934.

63. Sherman, H. C. and Smith, S. L.
The vitamins. 2d ed. The Chemical Catalog Co., New York. 1931.

64. Simmonds, Nina, Becker, J. E. and McCollum, E. V.
The distribution of vitamin E. J. Nutrition 1: 39-47. 1928.

65. Simonnet, H.
Les vitamines B. Z. Vitaminforsch. 2: 28-47. 1933.

66. Simonnet, H.
Les vitamines B. Z. Vitaminforsch. 2: 94-109. 1933.

67. Smith, A. H. and Bing, F. C.
Improved rate of growth of stock albino rats.
J. Nutrition 1: 179-189. 1928.

68. Sure, Barnett.
Dietary requirements for reproduction. I. The nutritive value of milk proteins from the standpoint of reproduction. J. Biol. Chem. 58: 681-692. 1924.

69. Sure, Barnett.
Dietary requirements for reproduction. II. The existence of a specific vitamin for reproduction.
J. Biol. Chem. 58: 693-709. 1924.

70. Sure, Barnett.
Dietary requirements for reproduction. III. The existence of the reproductive dietary complex (vitamin E) in the etherial extracts of yellow corn, wheat embryo, and hemp seed. J. Biol. Chem.
62: 371-396. 1924.

71. Sure, Barnett.
Dietary requirements for reproduction. IV. Solubility of the reproductive dietary complex (vitamin E) in various organic solvents.
J. Biol. Chem. 63: 211-223. 1925.

72. Sure, Barnett.
Dietary requirements for reproduction. V. The role of various vegetable and fruit oils in fertility and lactation. J. Biol. Chem. 69: 29-40. 1926.

73. Sure, Barnett.
Dietary requirements for reproduction. VI. Types of sterility produced on a skimmed milk powder reproduction-deficient diet. J. Biol. Chem.
69: 41-52. 1926.

74. Sure, Barnett.
Dietary requirements for reproduction. VII. The existence of a lactation-promoting factor in the unsaponifiable matter from wheat oil. J. Biol. Chem.
69: 53-74. 1926.

75. Sure, Barnett.
Dietary requirements for reproduction. VIII. Further studies of a skimmed milk powder reproduction-deficient diet. J. Biol. Chem. 74: 37-44. 1927.

76. Sure, Barnett.
Dietary requirements for reproduction. IX. Cod liver oil versus wheat oil as sources of vitamin E. J. Biol. Chem. 74: 45-53. 1927.

77. Sure, Barnett.
Dietary requirements for reproduction. XI. The potency of butter fat in vitamin E. J. Biol. Chem. 74: 71-84. 1927.

78. Sure, Barnett.
Dietary requirements for fertility and lactation. XIV. A quantitative biological method for the study of vitamin B requirements for lactation. J. Biol. Chem. 76: 673-683. 1928.

79. Sure, Barnett.
Dietary requirements for fertility and lactation. XV. The inefficiency of the lactating mother (Mus norvegicus albinus) in secreting vitamin B in the milk, and the relation of this phenomenon to infant mortality. (Detailed report). J. Biol. Chem. 76: 685-700. 1928.

80. Sure, Barnett.
Dietary requirements for fertility and lactation. XX. A differentiation of the vitamin B complex in rice polishings as evidenced in studies of lactation. J. Biol. Chem. 80: 297-307. 1928.

81. Sure, Barnett and Walker, D. J.
Dietary requirements for fertility and lactation. XXIII. The specific effect of vitamin B on lactation. J. Biol. Chem. 91: 69-75. 1931.

82. Taylor, M. W.
Reproduction and lactation on simplified diets. Unpublished thesis. Library, Iowa State College, Ames, Iowa. 1930.

83. Tso, Ernest.
The effects of synthetic diets on fertility and lactation. Proc. Soc. Exptl. Biol. Med. 24: 465-468. 1927.

84. Whitsitt, M. L.
Vitamin B (B_1) and G (B_2) contents of cotton seed products. Ind. Eng. Chem. 25: 1169-1171. 1933.

85. Wilkinson, P. D.
Diet in relation to reproduction and to rearing of young rats. Unpublished thesis. Library, Iowa State College, Ames, Iowa. 1930.

Table XIV

Reproduction using Sea Pure cod liver oil

Female number	% oil	Months on diet	Total No. of litters	Total No. of young	Cause of the death of the female
1	1	12	0	0	Killed
2	1	6	0	0	Unknown
3	1	12	0	0	Unknown
4	1	9	0	0	Killed
5	1	9	0	0	Killed
6	1	9	0	0	Killed
7	2	5	0	0	Pregnant
8	2	12	3	13	Killed
9	2	4	2	3	Unknown
10	2	9	2	7	Killed
11	2	3	4	4	Unknown
13	3	5	2	13	Parturition
14	3	5	0	0	Pregnant
15	3	6	1	4	Lost
16	3	9	0	0	Killed
17	3	9	0	0	Killed
18	3	9	0	0	Killed
19	3	8	0	0	Killed
20	3	8	0	0	Killed
21	3	8	0	0	Killed
22	3	8	0	0	Killed
23	4	4	0	0	Pregnant
24	4	5	0	0	Pregnant
25	4	5	0	0	Pregnant
26	4	3	0	0	Pregnant
27	4	3	1	4	Pregnant
28	4	9	1	3	Killed
29	5	6	2	13	Parturition
30	5	7	3	22	Pregnant
31	5	4	0	0	Pregnant
33	5	3	1	7	Pregnant
34	5	9	2	13	Killed
35	5	4	0	0	Pregnant
36	5	8	0	0	Ear Infection
37	5	8	1	2	Killed
38	5	8	2	7	Killed
39	5	6	0	0	Unknown
40	5	8	0	0	Killed
41	5	8	2	8	Killed
42	10	5	3	15	Pregnant
43	10	6	0	0	Pregnant
44	10	2	0	0	Pregnant
45	10	5	2	7	Pregnant
46	10	3	0	0	Pregnant

Table XV

Reproduction using Des Moines Drug oil

Female number	% oil	Months on diet	Total No. of litters	Total No. of young	Cause of the death of the female
47	1	10	0	0	Killed
48	1	10	0	0	Killed
49	1	10	0	0	Killed
50	1	10	0	0	Killed
51	1	10	0	0	Killed
52	2	10	1	2	Killed
53	2	10	1	3	Killed
54	2	5	0	0	Unknown
55	2	7	0	0	Pregnant
56	2	3	1	5	Unknown
58	3	5	0	0	Pregnant
59	3	12	4	30	Killed
60	3	12	3	18	Killed
61	3	7	0	0	Unknown
62	3	7	1	3	Pregnant
63	3	2	0	0	Unknown
64	3	6	2	11	Unknown
65	3	3	1	8	Parturition
66	3	8	1	8	Killed
67	3	3	0	0	Pregnant
68	3	3	0	0	Pregnant
69	4	12	1	1	Killed
70	4	2	0	0	Unknown
71	4	7	1	6	Parturition
72	4	2	0	0	Unknown
73	4	2	0	0	Pregnant
74	4	3	0	0	Unknown
75	4	3	0	0	Pregnant
76	4	9	3	15	Killed
77	4	6	3	14	Killed
78	5	5	1	5	Unknown
79	5	5	0	0	Pregnant
80	5	5	0	0	Pregnant
81	5	5	1	5	Unknown
82	5	9	1	5	Parturition
83	5	4	2	14	Killed
84	5	3	1	2	Parturition
85	5	4	0	0	Killed
86	5	3	0	0	Unknown
87	5	2	0	0	Pregnant
88	5	4	0	0	Parturition
89	5	8	0	0	Killed
90	5	3	0	0	Unknown
91	5	8	0	0	Killed
92	5	5	3	18	Parturition

Table XVI

Reproduction using Parke Davis oil

Female number	% oil	Months on diet	Total No. of litters	Total No. of young	Cause of the death of the female
94	1	9	0	0	Killed
95	1	4	0	0	Unknown
96	1	9	1	3	Killed
97	1	7	1	5	Killed
98	2	5	0	0	Pregnant
99	2	5	1	1	Pregnant
100	2	9	4	23	Unknown
101	2	9	1	6	Unknown
102	2	3	2	4	Unknown
103	3	8	1	5	Unknown
104	3	10	0	0	Killed
105	3	8	3	17	Pregnant
106	3	6	0	0	Pregnant
107	3	8	2	6	Unknown
108	3	2	0	0	Unknown
109	3	8	0	0	Unknown
110	3	4	2	9	Unknown
* 111	4	12	7	58	Killed
* 112	4	12	6	35	Killed
113	4	5	1	5	Pregnant
* 114	4	12	4	22	Killed
115	4	7	0	0	Unknown
* 116	4	6	1	3	Killed
117	5	10	1	6	Killed
118	5	5	1	6	Unknown
119	5	7	3	21	Unknown
120	5	6	0	0	Pregnant
121	5	4	2	8	Killed
122	5	4	0	0	Killed
123	5	5	0	0	Pregnant
124	5	4	0	0	Parturition
125	5	4	0	0	Pregnant
126	5	4	1	7	Pregnant
127	5	5	0	0	Pregnant
128	5	8	2	10	Unknown

* Lactation permitted during last four months on diet.

Table XVII

Reproduction using Fuller-Morrison oil

Female number	% oil	Months on diet	Total No. of litters	Total No. of young	Cause of the death of the female
129	1	10	0	0	Killed
130	1	10	0	0	Killed
131	1	7	0	0	Unknown
132	1	6	0	0	Killed
133	2	10	0	0	Killed
134	2	10	0	0	Killed
135	2	4	0	0	Pregnant
136	2	10	2	7	Killed
137	2	3	0	0	Killed
138	3	10	2	11	Killed
139	3	10	3	16	Killed
140	3	8	1	5	Unknown
141	3	5	1	1	Unknown
142	3	5	1	6	Unknown
143	3	3	1	3	Killed
144	3	3	0	0	Pregnant
145	3	8	1	6	Killed
146	3	8	1	4	Killed
147	3	3	0	0	Pregnant
148	4	10	1	1	Killed
149	4	4	0	0	Unknown
150	4	7	1	2	Unknown
151	4	6	1	5	Pregnant
152	5	6	3	19	Unknown
153	5	6	1	5	Unknown
154	5	11	3	20	Killed
155	5	11	1	3	Killed
156	5	11	2	5	Killed
157	5	3	0	0	Unknown
158	5	5	0	0	Pregnant
159	5	8	0	0	Killed
160	5	4	0	0	Pregnant
161	5	5	1	6	Parturition
162	5	8	2	12	Killed

Table XVIII

Reproduction using Eli-Lilly oil

Female number	% oil	Months on diet	Total No. of litters	Total No. of young	Cause of the death of the female
163	1	6	0	0	Killed
164	1	6	1	7	Unknown
165	1	6	1	2	Killed
166	1	6	1	2	Killed
167	2	4	1	6	Unknown
168	2	6	0	0	Unknown
169	2	4	0	0	Unknown
170	2	3	0	0	Unknown
* 171	3	8	3	13	Killed
* 172	3	8	2	16	Killed
* 173	3	7	3	20	Unknown
* 174	3	8	2	10	Killed
175	3	3	1	7	Parturition
176	3	4	2	11	Pregnant
177	3	5	2	11	Pregnant
178	3	4	3	17	Unknown
179	4	4	1	3	Killed
180	4	2	0	0	Unknown
181	4	4	0	0	Killed
182	4	4	0	0	Killed
183	5	5	2	11	Unknown
184	5	2	0	0	Pregnant
185	5	4	1	6	Killed
186	5	4	1	6	Killed
187	5	2	0	0	Parturition
188	5	8	2	11	Killed
189	5	4	0	0	Parturition
190	5	3	0	0	Parturition
191	5	7	3	10	Pregnant
192	5	3	0	0	Parturition

* Lactation permitted during last three months on diet.

Table XIX

Reproduction using Meads oil

Female number	% oil	Months on diet	Total No. of litters	Total No. of young	Cause of the death of the female
193	1	5	0	0	Killed
194	1	5	0	0	Killed
195	1	5	0	0	Killed
* 196	2	8	4	21	Unknown
197	2	3	1	2	Pregnant
198	2	2	1	6	Unknown
* 199	2	6	3	15	Killed
200	3	3	0	0	Lost
201	3	4	0	0	Unknown
202	3	5	0	0	Killed
203	3	5	0	0	Killed
204	3	3	1	7	Parturition
205	3	6	0	0	Unknown
206	3	2	1	6	Unknown
207	3	2	0	0	Unknown
208	4	2	0	0	Pregnant
* 209	4	8	3	21	Pregnant
210	4	8	2	9	Unknown
211	4	2	1	6	Unknown
212	5	2	1	6	Unknown
213	5	4	2	15	Pregnant
214	5	3	0	0	Pregnant
215	5	4	0	0	Killed
216	5	7	1	4	Pregnant
217	5	7	2	10	Pregnant
218	5	8	2	11	Pregnant
219	5	8	1	5	Unknown
220	5	8	0	0	Unknown

* Lactation permitted during last four months on diet.

Table XX

Reproduction using Marden-Wilde oil

Female number	% oil	Months on diet	Total No. of litters	Total No. of young	Cause of the death of the female
222	1	5	0	0	Killed
223	1	5	0	0	Killed
224	1	5	0	0	Killed
225	2	5	0	0	Killed
226	2	5	0	0	Killed
227	2	5	0	0	Killed
228	3	5	0	0	Killed
229	3	5	0	0	Killed
230	3	5	0	0	Killed
231	3	8	1	3	Killed
232	3	8	1	5	Killed
233	3	3	0	0	Unknown
234	3	8	0	0	Unknown
* 235	4	5	0	0	Killed
236	4	2	0	0	Unknown
237	4	5	0	0	Unknown
238	5	3	0	0	Pregnant
239	5	5	0	0	Killed
240	5	4	1	6	Pregnant
241	5	8	0	0	Killed
242	5	4	0	0	Tumor
243	5	8	0	0	Killed
244	5	6	0	0	Unknown
245	5	3	0	0	Ear Infection
246	5	8	1	1	Killed

* After four months on the diet shown above, female 235 was fed a diet in which 10% of Squibbs cod liver oil replaced the 4% of Marden-Wilde cod liver oil. In two months she gave birth to three litters. She was permitted to raise the last litter of 15 young and succeeded in weaning 8 at an average weight of 31 grams.

Table XXI

Reproduction using Squibbs cod liver oil

	Female: number:	% Oil:	Months: on diet	Total No. of litters:	Total: No. of young:	Cause of the death of the female
	247	1	4	0	0	Pregnant
	248	1	9	1	5	Killed
	249	1	6	2	7	Parturition
	250	1	6	0	0	Killed
	251	1	6	0	0	Killed
	252	1	6	0	0	Killed
	253	1	6	0	0	Killed
	254	1	6	0	0	Killed
	255	2	10	1	8	Killed
	256	2	10	0	0	Killed
	257	2	10	0	0	Killed
	258	2	9	2	9	Pregnant
*	259	2	8	3	15	Killed
*	260	2	8	4	26	Killed
*	261	2	8	3	14	Killed
*	262	2	8	4	25	Killed
	263	3	10	3	15	Killed
*	264	3	10	4	27	Killed
	265	3	5	1	9	Pregnant
	266	3	6	1	9	Pregnant
*	267	3	8	3	26	Killed
*	268	3	8	3	20	Killed
*	269	3	8	2	15	Killed
*	270	3	8	2	11	Killed
	271	3	4	0	0	Parturition
	272	3	8	4	20	Parturition
	273	3	5	0	0	Pregnant
	274	3	4	1	4	Killed
*	275	4	12	2	14	Killed
	276	4	6	3	17	Pregnant
*	277	4	12	3	8	Killed
*	278	4	12	5	35	Killed
*	279	4	12	5	39	Killed
*	280	4	5	3	21	Killed
	281	4	3	1	5	Pregnant
	282	4	4	1	7	Pregnant
*	283	4	8	4	30	Killed
	284	4	2	1	9	Pregnant

* Lactation permitted during last four months on diet.

Table XXI continued

Reproduction using Squibbs cod liver oil

	Female number	% oil	Months on diet	Total No. of litters	Total No. of young	Cause of the death of the female
*	285	5	9	6	48	Killed
*	286	5	9	3	20	Killed
*	287	5	10	6	28	Killed
*	288	5	8	4	28	Killed
*	289	5	8	3	17	Pregnant
*	290	5	8	2	24	Killed
	291	5	4	2	8	Parturition
*	292	5	8	4	16	Killed
*	293	5	8	5	32	Killed
	294	5	3	1	1	Parturition
*	295	5	8	2	19	Killed
	296	5	8	5	18	Killed
	297	5	8	1	2	Killed
	298	5	8	3	13	Parturition
	299	5	4	0	0	Pregnant
	300	5	8	2	6	Ear Infection
	301	5	4	1	7	Parturition
	302	10	8	6	25	Parturition
	303	10	4	2	5	Unknown
	304	10	12	3	21	Killed
	305	10	9	5	25	Parturition
	306	10	12	4	21	Killed

* Lactation permitted during the last four months on diet.

Table XXII

Reproduction using diets 43, 44, 45 and 46.
For composition of these diets see page 42.

Female number	% oil	Months on diet	Total No. of litters	Total No. of young	Cause of the death of the female
Diet 45. Extracted wheat embryo 12% plus Squibbs oil 5%.					
* 310	5	8	8	60	Killed
* 311	5	8	4	25	Killed
* 312	5	8	5	37	Killed
* 313	5	8	4	32	Killed
* 314	5	10	4	24	Killed
* 315	5	10	4	22	Killed
* 316	5	10	5	37	Killed
Diet 46. Natural wheat embryo 12% plus Squibbs oil 5%					
* 317	5	10	4	28	Killed
* 318	5	10	4	18	Killed
* 319	5	10	3	13	Killed
Diet 43. Yeast 12% plus butter fat 5% plus Squibbs oil 0.5%.					
320	0.5	5	0	0	Killed
321	0.5	5	0	0	Killed
322	0.5	4	1	6	Unknown
323	0.5	4	0	0	Killed
Diet 44. Yeast 12% plus butter fat 5% plus Squibbs oil 0.5%.					
324	0.1	5	0	0	Killed
325	0.1	5	0	0	Killed
326	0.1	5	0	0	Killed
327	0.1	5	0	0	Killed

* Lactation permitted during last four months on diet.

Table XXIII

Reproduction using the growing ration

Female number	Months on diet	Total No. of litters	Total No. of young	Cause of the death of the female
328	5	3	26	Killed
* 329	8	6	52	Killed
* 330	8	4	37	Killed
* 331	8	5	38	Killed
332	5	2	15	Unknown
333	11	4	29	Killed
334	12	11	85	Killed
335	12	3	10	Killed
336	12	7	62	Killed
337	8	2	12	Unknown
338	12	12	99	Killed
339	12	6	35	Killed
340	12	7	52	Killed
341	12	9	83	Killed
342	12	12	98	Killed
343	12	7	55	Killed
** 344	10	3	20	Killed
** 345	10	5	29	Killed
** 346	10	5	43	Killed
** 347	10	4	37	Killed
** 348	10	6	46	Killed
** 349	10	5	37	Killed

* Lactation permitted during last four months.

** Lactation permitted during the entire ten months.

Table XXIV

Reproduction following changes of diet

Female number	Old diet number	New diet Number	Months on new diet	Young on old diet	Young on new diet
47	7	47	3	0	0
48	7	47	3	0	13
49	7	47	3	0	9
50	7	47	3	0	7
51	7	47	3	0	0
52	8	47	3	2	0
53	8	47	3	3	9
94	12	47	3	0	0
96	12	47	3	3	8
97	12	47	3	5	8
129	17	47	3	0	11
130	17	47	3	0	8
132	17	47	3	0	6
133	18	47	3	0	9
134	18	47	3	0	0
136	18	47	3	7	0
137	18	47	3	0	7
222	32	42	3	0	10
223	32	42	3	0	0
224	32	42	3	0	0
225	33	42	3	0	0
226	33	42	3	0	0
227	33	42	3	0	0
228	34	42	3	0	0
229	34	42	3	0	0
230	34	42	3	0	0
235	35	42	3	0	15
248	37	47	3	5	7
250	37	47	3	0	5
255	38	47	3	8	8
256	38	47	3	0	0
257	38	47	3	0	0
320	43	42	3	0	0
321	43	42	3	0	4
323	43	42	3	0	2
324	44	47	3	0	0
325	44	47	3	0	0
326	44	47	3	0	10
327	44	47	3	0	11

Table XXV

Lactation using different cod liver oils.

Female number	Diet No.	Months on lactation	Number of young	Young weaned	Average weaning weight	% mortality
76	10	3	14	11	37	21
77	10	3	14	6	46	57
111	15	4	30	11	30	63
112	15	4	10	7	43	30
114	15	4	12	5	44	58
116	15	4	3	3	30	0
171	24	3	11	4	48	64
172	24	3	8	2	25	75
173	24	3	7	1	25	89
174	24	3	9	3	30	33
196	28	4	5	3	57	40
199	28	4	13	1	32	93
209	30	4	19	3	47	84
259	38	4	15	11	49	27
260	38	4	10	3	47	70
261	38	4	21	16	48	24
262	38	4	17	13	49	24
264	39	4	9	8	43	11
267	39	4	26	16	50	39
268	39	4	20	19	53	5
269	39	4	15	13	49	13
270	39	4	11	8	48	27
275	40	4	8	8	44	0
277	40	4	6	2	45	66
278	40	4	23	15	47	35
279	40	4	24	22	41	8
280	40	4	21	14	47	33
283	40	4	24	15	43	38

Table XXV continued

Lactation using different cod liver oils

Female Number	Diet No.	Months on lactation	Number of young	Young weaned	Average weaning weight	% mortality
285	41	4	25	21	50	16
286	41	4	4	4	45	0
287	41	4	23	14	49	39
288	41	4	23	23	48	0
289	41	4	13	13	40	0
290	41	4	24	20	41	17
292	41	4	12	7	50	42
293	41	4	16	10	47	25
295	41	4	19	18	50	5
310	45	4	30	13	46	57
311	45	4	11	3	50	73
312	45	4	22	22	45	0
313	45	4	8	8	40	0
314	45	4	24	11	46	54
315	45	4	22	18	32	18
316	45	4	37	32	44	13
317	46	4	28	5		100
318	46	4	9	9	52	0
319	46	4	13	3	25	80

Table XXVI

Lactation using diets containing growing ration

Female number	Diet number	Months on diet	Litters	Young born	Young weaned	Average weight	% Mortality
350	50	5	0	0	0		
351	50	2	0	0	0		
352	50	12	0	0	0		
353	51	15	0	0	0		
354	51	15	0	0	0		
355	51	12	0	0	0		
356	52	15	0	0	0		
357	52	10	0	0	0		
358	52	15	0	0	0		
359	53	4	0	0	0		
360	53	2	0	0	0		
361	53	15	3	21	0		100
362	54	15	5	32	1	40	94
363	54	15	4	26	2	21	93
364	54	15	5	30	0		100
365	55	5	2	10	0		100
366	55	15	7	44	10	30	79
367	55	15	5	34	9	30	74
371	57	12	5	35	21	45	40
372	57	15	6	40	18	36	55
373	57	15	2	16	16	35	0
374	58	15	4	34	30	42	12
375	58	15	3	25	25	49	0
376	58	15	2	17	16	48	6
377	59	15	7	48	46	51	4
378	59	15	6	39	33	48	15
379	59	15	5	37	35	51	11
380	59	10	5	39	15	36	62
381	59	10	4	20	20	52	0
382	59	10	4	24	22	57	8
332	60	5	2	15	13	42	13
333	60	11	4	29	25	46	14
405	60	10	5	33	16	45	51
406	60	10	5	38	38	45	0
407	60	10	4	37	25	37	32
329	60	5	3	28	22	41	21
330	60	4	1	11	6	51	45
331	60	4	1	2	2	30	0

Table XXVI continued

Lactation using diets containing growing ration

Female number	Diet number	Months on diet	Litters	Young born	Young weaned	Average weight	% Mortality
344	60	10	3	20	13	40	35
345	60	10	4	25	24	46	4
346	60	10	5	34	33	46	3
347	60	10	3	21	18	52	14
348	60	10	5	29	11	34	62
349	60	10	5	36	35	47	3
408	61	10	6	46	26	35	44
409	61	10	4	28	25	49	11
410	61	10	5	38	35	42	8
411	62	10	2	9	4	62	55
412	62	10	4	28	26	56	7
413	63	10	3	28	19	61	32
414	63	10	4	37	23	47	37
415	63	10	4	39	22	53	44
416	64	10	3	28	28	51	0
417	64	10	3	29	13	48	55
418	64	10	3	23	23	52	0

Table XXVII

* Lactation using diets containing individual components of the growing ration with and without supplements of vitamins B and G.

Diet number	Number of females	Young born	Young after 7 days	Young weaned	% mortality after 7 days	Average wt. at weaning. 28 days. Gms.
10				Wheat		
101	6	35	33	12	64	15
102	6	36	33	10	69	19
103	6	35	34	12	65	21
104	6	31	28	17	39	26
105	6	36	28	19	32	21
106	5	30	30	24	20	48
107	6	36	28	28	0	58
108	6	36	35	34	3	41
				Alfalfa		
111	6	34	34	10	71	19
112	6	36	30	23	23	24
113	6	35	26	15	42	18
114	6	33	20	14	30	15
115	6	36	28	17	39	22
116	6	36	36	24	33	39
117	6	34	26	26	0	46
118	6	36	34	28	18	28
				Tankage		
121	6	36	32	0	100	
122	6	35	20	0	100	
123	6	36	34	0	100	
124	6	36	29	0	100	
125	6	35	29	20	31	12
126	6	36	36	10	72	16
127	6	36	29	26	11	32
128	6	36	33	30	9	35

* The composition of these diets appears in table XXVIII.

Table XXVII continued

* Lactation using diets containing individual Components of the growing ration with and without supplements of vitamins B and G.

Diet number	Number of females	Young born	Young after 7 days	Young weaned	% mortality after 7 days	Average wt. at weaning. 28 days. Gms.
			Butter milk powder			
131	6	36	35	6	83	19
132	6	36	34	4	88	25
133	6	36	34	0	100	
134	6	35	19	5	74	33
135	6	36	36	30	17	31
136	6	36	34	18	44	27
137	6	36	30	29	3	53
138	6	36	36	35	3	49
			Fish meal			
141	6	36	25	0	100	
142	6	36	28	5	82	26
143	6	35	34	3	91	17
144	6	36	30	6	80	22
145	6	36	36	15	58	33
146	6	36	35	0	100	
147	6	36	35	35	0	57
148	6	36	35	35	0	48
			Linseed oil meal			
151	6	36	21	5	76	17
152	6	36	29	17	41	21
153	6	36	36	5	86	21
154	6	36	27	0	100	
155	6	36	35	28	20	24
156	6	36	35	35	0	35
157	6	36	35	35	0	49
158	6	32	32	29	9	50

* The composition of these diets appears in table XXVIII

Table XXVII continued

* Lactation using diets containing individual components of the growing ration with and without supplements of vitamins B and G.

Diet number	Number of females	Young born	Young after 7 days	Young weaned	% mortality after 7 days	Average wt. at weaning. 28 days. Gms.
			Cotton seed meal			
161	6	36	23	4	83	22
162	6	36	12	6	50	30
163	6	36	21	2	80	16
164	4	24	24	4	83	18
165	6	36	35	35	0	32
166	6	36	29	26	12	55
167	6	36	33	33	0	53
168	6	36	32	30	6	43
			Growing ration			
175	6	36	35	35	0	48
176	6	36	32	28	12	49
177	6	36	33	32	3	49
178	6	36	36	32	11	53
			Synthetic			
187	6	36	32	29	9	45
188	6	36	29	18	38	40
188	4	24	18	12	33	50

* The composition of these diets appears in table XXVIII.

Table XXVIII

Composition of diets 101-188 inclusive

Final numeral in diet number	% of added component	Added component 25% + vitamin supplements shown below
1	10	
2	25	
3	40	
4	60	
5	25	Vitamin B
6	25	Vitamin G (liver)
7	25	Vitamins B and G (liver)
8	25	Vitamins B and G (yeast)

Wheat diets are shown below as an example.

Diet number	% wheat	Vitamin supplements
101	10	
102	25	
103	40	
104	60	
105	25	Vitamin B
106	25	Vitamin G (liver)
107	25	Vitamins B and G (liver)
108	25	Vitamins B and G (yeast)